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16. ABSTRACT

The objective of this research was to measure the fate and mobility of organic mutagens in municipal sludge amended soil. Initially, seventeen municipal sewage sludge samples were collected. The mutagenic potential of 49 separate fractions extracted from 17 samples of municipal sewage sludge was determined using the Salmonella/ microsome assay. None of the fractions extracted from four of the sludges induced a positive response in the bioassay. Twenty-six fractions failed to induce a doubling of revertant colonies at two consecutive dose levels, while seventeen fractions induced a moderate mutagenic response (35 to 100 net revertants 10 per milligrams), and six induced a relatively high mutagenic response (greater than 100 net revertants per 10 milligrams). The maximum specific activity was 187 net revertants per 10 mg induced by the methanol fraction extracted from the AD2 sludge with metabolic activation; while the maximum weighted activity was 1197 revertants per gram of sludge induced by the combined fraction of the SM4 sludge. For the sludge fractions tested, strain TA98 was the most sensitive, and the majority of fractions required metabolic activation to induce a positive response.

One sludge sample which induced a strong positive response was selected for use in a field study. Sludge samples were collected and applied at three application rates to undisturbed soils enclosed in lysimeters to monitor the fate and mobility of sludge borne mutagens in soil. The maximum specific activity of the sludge amended soil was 416 net revertants per 10 milligrams of residue induced by the methanol fraction extracted from the Weswood soil collected 154 days following sludge application (totaling 150 Mg/ha). The mutagenic potential of the sludge amended soil was observed to decrease slowly with time. A positive response was still observed for several of the fractions extracted from the sludge amended soil collected 207 days after sludge application. The mutagenic activity per unit weight of soil decreased to near background levels over the course of the study for only one treatment (Padina sand with 50 Mg/ha). Eight of twenty-seven leachate samples from the waste amended soils induced a positive mutagenic response; while twelve samples induced a weighted activity of greater than 100 revertants per liter. The maximum specific activity of the acetone extractable organics recovered from the leachate water was 207 net revertants per 10 milligrams of residue induced without metabolic activation by the leachate water from the Weswood soil which received two sludge applications totaling 150 Mg/ha.

None of the extracts from plants grown on the sludge amended soil induced a positive response in the bioassay. When the interactions of the plant extracts were evaluated in the presence of positive controls or the extract of the sludge, synergistic, antagonistic, and additive interactions were observed. However, the extract of the plants grown on the sludge amended soil were observed to inhibit the mutagenic activity of the sludge extract.

The results indicate that municipal sludges may contain a persistent mutagenic residue. In addition, leaching of mutagens may be a serious problem in coarse textured soils, or in areas which receive high amounts of rainfall. Uptake of mutagens into plants does not appear to be a significant problem for the sludges, soils, plants, and application rates employed, as none of the extracts of plants grown on sludge amended soil induced a positive response.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and nonionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

Disposal of municipal sewage sludge in a safe and economical manner is a major environmental concern presently facing the Agency., One disposal option currently in practice is agricultural land application, designed to provide for recycling of water and nutrients. There are, however, sludge contaminants which may be associated with health risks. This project was designed to measure the levels, stability and mobility of the mutagenic components of land applied sludge, and whether these components were taken up in active form by plant life.

Lawrence W. Reiter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

The objective of this research was to measure the fate and mobility of organic mutagens in municipal sludge amended soils. Initially, seventeen municipal sewage sludge samples were collected. The mutagenic potential of 49 separate fractions extracted from 17 samples of municipal sewage sludge was determined using the Salmonella/ microsome assay. None of the fractions extracted from four of the sludges induced a positive response in the bioassay. Twenty-six fractions failed to induce a doubling of revertant colonies at two consecutive dose levels, while seventeen fractions induced a moderate mutagenic response (35 to 100 net revertants per 10 milligrams), and six induced a relatively high mutagenic response (greater than 100 revertants per 10 milligrams). The maximum specific activity was 187 net revertants per 10 mg induced by the methanol fraction of the AD2 sludge with metabolic activation; while the maximum weighted activity was 1197 revertants per gram induced by the combined fraction of the SM4 sludge. For the sludge fractions tested, strain TA98 was the most sensitive, and the majority of fractions required metabolic activation to induce a positive response.

One sludge sample which induced a strong positive response was selected for use in a field study. Sludge samples were collected and applied at three application rates to undisturbed soil lysimeters to monitor the fate

and mobility of sludge borne mutagens in soil. The maximum specific activity of the sludge amended soil was 416 net revertants per 10 milligrams induced by the methanol fraction extracted from the Weswood soil collected 154 days following sludge application (totalling 150 Mg/ha). The mutagenic potential of the sludge amended soil decreased slowly with time. A positive response was still observed for several of the fractions extracted from the sludge amended soil collected 207 days after sludge application. The mutagenic activity per unit weight of soil decreased to near background levels over the course of the study for only one treatment (Padina sand with 50 Mg/ha). Eight of twenty-seven leachate samples from the waste amended lysimeters were found to contain detectable levels of organic mutagens. The maximum specific activity of the acetone extractable organics recovered from the leachate water was 207 net revertants induced without metabolic activation by the leachate water from the Weswood soil which received two sludge applications totalling 150 Mg/ha.

None of the extract from the plants grown on the sludge amended soil induced a positive response in the bioassay. When the interactions of the plant extract were evaluated in the presence of positive controls or the extract of the sludge, synergistic, antagonistic, and additive interactions were observed. However, the extract of the plants grown on the sludge amended soil were observed to inhibit the mutagenic activity of sludge extracts.

The results indicate that some municipal sludges may contain a persistent mutagenic residue. In addition, leaching of mutagens may be a problem in coarse textured soils, or in areas which receive high amounts of rainfall. None of the extracts from plants grown in this study exhibited mutagenic activity, indicating that the food chain is protected by lack of transfer to the vegetative portions of the plant, or that other plant constituents inhibited the activity of any mutagens which may have been taken up.

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SECTION 1

INTRODUCTION

From the time man began to congregate in urban centers, the safe and economical disposal of municipal sewage has been a major concern. More than 120 years ago, Daubeny (1860) stated that "from the disposal of the sewerage of London alone we are incurring a national loss of more than a million sterling; ...it is of vital importance for the nation at large to use every effort in its power to find some useful application for its excrementitious matter." Given the present status of rising fertilizer costs and water shortages, it is of vital importance to our nation to use every effort to recycle the nutrients and water present in municipal sewage.

More than 8 billion gallons of municipal sewage are treated in the United States each day (NIEHS, 1977). Treatment of municipal sewage results in the generation of approximnately 7 million tons of dry sludge each year (EPA, 1983). Land application is one potential disposal method for the sludge from municipal sewage treatment plants. Land treatment is designed to provide for the recycling of water and nutrients, and the retention or degradation of organics in the surface layer of soil. A survey of 1008 publicly owned treatment works found 40% of the total sludge generated was to be used in some form of land application (EPA, 1983).

Health risks which may be associated with land application of sludge include pathogens (e.g., bacteria, viruses, protozoa and helminths), trace elements (e.g., cadmium, lead, and zinc), nitrates and organics (Kowal, 1985). While much information exists pertaining to the environmental fate of specific pathogens (Gerba and McNabb, 1981; Feacham et al., 1978; Yeager and O'Brien, 1979; Wellings et al., 1975; Lefler and Kott, 1974; Hays, 1977), heavy metals (Chaney, 1980; Chang and Page, 1978; Brown, 1978), and nitrate (Hossner et al., 1978; Brown et al., 1983), much less is known about the fate and composition of organic chemicals in municipal sewage (Kowal, 1985).

A partial list of organic chemicals which have been identified in municipal wastewater or sludge is provided in Table 1-1. This list includes a variety of chemical classes such as chlorinated solvents, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons. Several of the chemicals listed in Table 1-1 have been found to induce increased frequencies of mutations in prokaryotic or eukaryotic systems (McCann et al., 1975; Scott et al., 1982), which may indicate that these chemicals could also induce cancer and other genotoxic effects in mammalian systems (Ames and McCann, 1975). There are four categories of test systems which could be used to measure the genotoxic potential of an environmental sample including epidemiologic studies, animal feeding studies, tissue culture, and short-term microbial assays. Of these procedures, only the microbial assays offer a rapid and inexpensive tool which can be used to monitor the genotoxic potential of a large number of environmental samples.

TABLE 1-1. PARTIAL LIST OF ORGANIC COMPOUNDS IDENTIFIED IN SAMPLES OF MUNICIPAL SEWAGE SLUDGE

Compound	Concentration Range (mg/kg)	Carcino-genicity[1]	Muta-genicity[2]	Muta-genicity[3]	Reference
Acrylonitrile	.04-82	+	+	+	Jacobs *et al.*, 1978
Chlorobenzene	2.1-846	0	0	+	"
p-chlorotoluene	1.1-324	0	0	0	"
1,2-dichlorobenzene	.03-809	0	0	+	"
1,3-dichlorobenzene	.02-1650	0	0	+	"
Ethylbenzene	1.2-66	0	0	0	"
Hexachloroethane	.0004-17	+	0	0	"
Styrene	.12-5850	0	–	0	"
Tetrachloroethane	.00001-0.12	+	–	–	"
1,2,3-trichlorobenzene	.006-51.2	0	0	+	"
1,3,5,trichlorobenzene	.005-39.7	0	0	+	"
Aldrin	.013-0.1	0	–	0	Saleh *et al.*, 1980
Dieldrin	.003-.032	+	–	0	"
DDT	.006-.011	+	–	0	"
DDE	.011	+	–	0	"
DDD	.006-.048	+	–	0	"
2,4-D	7.6-41	0	0	+	"
Dieldrin	<03-0.44	+	–	0	Furr *et al.*, 1976
PCB's	<.01-23	+	–	+	"
Fluoranthene	1-334	–	–	–	Bridle and Webber, 1983
Fluorene	0.8-115	0	0	0	"
Benzo(a)pyrene	34	+	+	+	"
Anthracene	8-311	0	0	0	"
Chrysene	<600	+	+	+	Webber *et al.*, 1982
Pyrene	<600	–	–	–	"
Benzo(a)pyrene	<600	+	+	+	"
Dibutylphthalate	60-500	+	+	+	Strachan *et al.*, 1983

TABLE 1-1 CONTINUED

Compound	Concentration Range (mg kg)	Carcino-genicity[1]	Muta-genicity[2]	Muta-genicity[3]	Reference
Di(2-ethylhexyl)-phthalate	30-130	+	-	0	Strachan *et al.*, 1983
Lindane	.006-.003	+	-	0	Majeti and Clark, 1981
Heptachlor	.001-.021	+	-	0	"
Chlordane	.008-.030	0	0	0	"
PCB	.07-1.1	+	-	+	"

1 - Carcinogencity as determined from limited review of literature. + = positive; - = negative; 0 = unknown.

2 - Mutagenicity as measured in standard plate incorporation with *S. typhimurium*. + = positive; - = negative; 0 = unknown.

3 - Foreward mutations as measured in methionine assay with *A. nidulans*. + = positive; - = negative; 0 = unknown.

Care must be taken in the disposal of municipal sewage to prevent the transmission of genotoxic compounds to the human population. In a properly managed land treatment system, organic mutagens in a sludge amended soil may be transformed, degraded or immobilized. Under conditions of mismanagement or environmental stress, organic mutagens may be released from the facility by volatilization, leaching into groundwater, runoff into surface waters, or plant uptake. The present study was conducted to evaluate the fate of organic mutagens in a land application system.

The first objective of this research was to evaluate the bacterial mutagenicity and organic chemical characteristics of municipal sewage sludge. More than ten municipal sludges were selected for evaluation to represent a range of domestic and industrial contributions. In addition, one treatment plant was selected from which multiple samples were collected over a ten month period to provide preliminary data on the variability of mutagenic constituents in sludge from a single source. The primary objective of this research project was to monitor the mobility and stability of mutagens in municipal sludge amended soil. To evaluate stability, sludge was applied on three separate occasions at two application rates to 18 undisturbed monolith lysimeters. Soil samples were collected from the sludge amended and control lysimeters for 180 days following sludge application. To evaluate mobility, leachate water was collected from suction cups installed 75 cm below the zone of incorporation. Finally, a limited study was conducted in the greenhouse to evaluate the potential for translocation of mutagens into alfalfa and bermudagrass grown on sludge amended soil.

SECTION 2

CONCLUSIONS

1. The bacterial mutagenicity of municipal sewage sludges exhibits tremendous variation both between different sources and from a single source.

2. The specific activity of several sludge fractions was not appreciably greater than the solvent control. For the mutagenic sludges, the maximum specific activity was 188 net revertants per 10 milligrams for the methanol fraction from the AD2 sludge with metabolic activation; and, the maximum weighted activity was 1197 net revertants per gram of sludge for the combined fraction of the SM4 sludge.

3. There does not appear to be a correlation between the level of mutagenicity in sludge, and the level of industrial contribution; however, this correlation may be more direct if it were possible to base this comparison on the specific type of industrial contribution.

4. For all of the sludges tested, strain TA98 appeared to be the most sensitive strain.

5. Although a limited number of sludge fractions conained direct-acting mutagens, for the majority of sludge fractions mutagenicity was only observed in the presence of metabolic activation.

6. For the sludges and soils studied, the specific activity of the sludge amended soil increased following application, and required approximately six months to reach a maximum before exhibiting signs of detoxification. In addition, selected fractions of samples collected 207 days following sludge application continued to induce a response that was greater than twice the concurrent solvent control.

7. For the majority of treatments evaluated, the weighted activity of the sludge amended soil was unchanged or slightly reduced for as long as 207 days following sludge application. However, for the fractions extracted from the Padina sand which received one sludge application at a rate of 50 mT/ha, the weighted activity of the sludge amended soil collected 175 days after application was approximately equal to the weighted activity of the control soil.

8. Several samples of leachate water from the sludge amended soil were found to contain appreciable levels of organic mutagens. Although the chemical constituents of these samples were not identified, the results do indicate that mutagenic chemicals can migrate below the zone of sludge incorporation.

9. None of the extracts of plant samples tested induced a mutagenic response. Although these results suggest that translocation of mutagens did not occur, inhibition of mutagens by chlorophyl in the plants may have inhibited the activity of any mutagenic chemicals which were present in the plant extracts.

SECTION 3

RECOMMENDATIONS

1. Although bacterial mutagens were detected in most of the sludges tested, a more accurate description of the genotoxic potential of the municipal sludges would be obtained if additional testing were conducted using assays to measure DNA repair and chromosome damage, and these results were combined with a chemical analysis.
2. Short-term bioassays should be utilized as a tool for determining the half-life of mutagens in sludge amended soils.
3. The extraction procedure used for sludge and soil samples in this study is useful for environmental monitoring; however, a more detailed fractionation procedure would be valuable to isolate the most active mutagens in the sludge.
4. Since the sludges tested in the lysimeter study contained a persistent and highly mutagenic residue, land application of municipal sludges with a relatively high mutagenic potential should be conducted at application rates that are sufficiently low as to prevent migration or accumulation of mutagenic constituents.
5. The rate of sludge application should be carefully monitored and regulated to prevent sludge borne mutagens from leaching into the subsurface environment.

6. Although plant uptake of sludge borne mutagens does not appear to be a serious problem for the sludges, soils and environmental conditions evaluated in this study, the use of sludge amended soils for the production of food chain crops should be restricted and carefully monitored.

7. Additional research is needed to evaluate the interactions of climatic conditions and soil texture, especially as it affects degradation and leaching of mutagens in sludge amended soil.

SECTION 4

BIOLOGICAL ANALYSIS

The analytical procedure which has been employed in this research to measure the potential genetic toxicity of sludge and environmental samples is the Salmonella/microsome assay of Ames *et al.*, 1975). This technique measures the mutagenic effects of a test substance by comparing its effect on a microorganism with that of standard substances with known toxicological effects. The test typically employs four specially constructed strains of *Salmonella typhimurium* to detect point mutations in a haploid genome. Since the composition of DNA is essentially the same in all organisms, an agent which induces mutations in a microorganism is also assumed to be capable of mutating mammalian DNA. The major differences separating bacterial mutagens and mutagens active in mammalian systems are a result of differences in metabolism, distribution and excretion. The validity of using a bacterial mutagenicity assay for the detection of potential carcinogens has been enhanced by the finding that a mutation is the first step in transformation of a human lung cell into a malignant state (Santos *et al.*, 1985). Validation studies have indicated that the accuracy of the Salmonella/microsome assay may be as high as 85 to 90%

(McCann et al., 1975), or as low as 58% (Rinkus and Legator, 1978). More recent evaluations suggest that the accuracy may be as high as 90% if based on significant positive carcinogens (Claxton et al., 1986) or trans-sex/species carcinogens (Tennant et al., 1986). In the present study, the Salmonella/microsome assay has been used as a rapid and inexpensive tool for monitoring the fate of organic mutagens in a municipal sludge amended soil. The following discussion presents a description of the test system, the standard protocols utilized in this research, and an evaluation of the variability of the assay for the various positive and negative controls.

MATERIALS AND METHODS

Laboratory Mutagenicity Bioassays

A microbial test system was used to ascertain the mutagenic effects of model compounds (positive and solvent controls) and organic mixtures present in the extracts of field samples. This test was used because it has been validated extensively with more than 5,000 different test chemicals (EMIC, 1985).

This test system uses a prokaryotic organism to detect point mutations. The Salmonella/microsome assay (Ames et al., 1975) measures the ability of a sample to induce reverse mutations in selected strains of Salmonella typhimurium. This system is a good predictor of the carcinogenicity of organic compounds. This test system found mutagenic activity for 90% of the carcinogens tested in an assay of 300 chemicals (McCann et al., 1975). In a comparative evaluation of six different short-term bioassays, the Salmonella assay correctly identified 93% of the organic carcinogens studied (Purchase et al., 1976). A more recent review by Rinkus and Legator (1979) found that 94% of the high activity carcinogens from fifteen chemical classes were detected as mutagens in the *Salmonella* assay with an overall correlation of 77% when 465 chemicals from 39 classes were included. Rinkus and Legator (1979) also noted a high specificity for the *Salmonella* assay, with only 13% of the non-carcinogens tested being mutagenic in *Salmonella*. Classes of chemicals which are poorly detected in the *Salmonella* assay include carbamyls, phenyls, bactericides, halogenated compounds, steroids, hydrazines and chloroethylenes. More recently, Tennant *et al*. (1986) observed that short-term genotoxicity bioassays do appear to be a good indicator of carcinogens which can induce tumor formation in both sexes of two different species of laboratory animals. They suggest that the genotoxicity assays are sensitive to those chemicals which are more likely to have the most direct implication for human health effects. In addition, Claxton *et al*. (1986) also observed that the sensitivity of this assay was very high when evaluated using the strong positive carcinogens.

Bioassay Procedures

The *Salmonella*/microsome assay of Ames *et al*. (1975) was used to evaluate the mutagenicity of the solvent extractable organic compounds in the fractions of sludge or sludge amended soil. *Salmonella* strains were kindly supplied by Dr. B.N. Ames (University of California, Berkeley, CA). The procedural methods were the same as Ames *et al*. (1975) with the modifications suggested by Maron and Ames (1983). The *Salmonella* tester strain TA98 was utilized with and without metabolic activation. Selected samples were also tested with strains TA100, TA1538 and TA1535. A description of the genotype of the four tester strains used in this study is provided in Table 4-1. The S9 mixture (9,000 x g supernatent from homogenized liver) contained 0.3 mL rat-liver S9 and 0.7 mL cofactor supplement (11.4 mM $MgCl_2$, 47 mM KCl, 7.1 mM glucose-6-phosphate, 5.7 mM NADP and 140 mM potassium phosphate buffer at pH 7.4). Aroclor induced Sprague-Dawley rat liver was obtained from Organon Teknika (West Chester, PA). The addition of mammalian metabolism to the assay system is designed to mimic the metabolism which would occur in an exposed mammalian population, and allows the detection of both direct and indirect acting mutagens. Chemicals which do not require metabolic activation to reach their ultimate mutagenic form are described as direct acting, while those which must be metabolized to reach their mutagenic form are described as indirect acting. Aroclor induced rat liver is used because previous research has indicated that this preparation efficiently activates the broadest range of chemicals of any inducer studied (Safe, 1984).

The extracts of the various environmental samples were tested in the standard plate incorporation assay at a minimum of four dose levels (mg/plate): 1.0, 2.5, 5.0, and 10.0. To conduct the assay, the specific strain to be tested with the sample extract is added to 2.5 mL of soft agar containing a low level of histidine and biotin along with the different doses of the test sample. A 100 µL suspension containing approximately 2 x 10^8 bacteria is added to the molten top agar at 47°C. A 50 µL solution containing the test chemical is then added to the top agar, and for tests using metabolic activation, a 500 µL solution of the S9 mix is also added. The top agar is then mixed on a vortex mixer, and plated on a minimal agar

TABLE 4-1. GENOTYPE OF THE *S. typhimurium* STRAINS USED FOR MUTAGEN TESTING

Histidine Mutation[1]		Additional Mutation[2,3]			
his G46	his D3052	LPS	Repair	R factor	Source
TA1535	TA1538	rfa	uvrB	-R	B. N. Ames
TA100	TA98	rfa	uvrB	+R	B. N. Ames

1. All strains originally derived from *S. typhimurium* LT-2.
2. rfa = deep rough characteristics, lack lipopolysaccharide cell wall.
3. uvrB = deletion through *uvrB* which includes nitrate reductase and biotin genes. Cells lack excision repair.

+R = resistance transfer factor, R = pKM101; enhanced sensitivity of plasmid strains may be due to increase in error-prone repair.

plate. The bacteria undergo several divisions with the reduced level of histidine, resulting in the formation of a light background lawn of growth on the petri dish and providing a route of exposure for the mutagens in the sample extract. The plates were incubated for 72 hours and cells reverting to the wild-type appeared as obvious large colonies on the plate. The number of colonies appearing on each plate was determined using a New Brunswick Biotran II automatic colony counter.

The data were analyzed using the modified two-fold rule (Chu *et al.*, 1981). Using this procedure, a response is considered positive only if the average response for at least two consecutive dose levels was greater than twice the average response for the concurrent negative/solvent control (Chu *et al.*, 1981).

All strains were checked monthly for nutritional markers (histidine and biotin) and other genetic characteristics, i.e., sensitivity to crystal violet, ampicillin, and UV light. On each test date, all tester strains were calibrated with positive and negative controls.

RESULTS AND DISCUSSION

The results presented in the following section represent the positive and solvent controls for the three years during which the research was conducted. Since testing was conducted using primarily strain TA98, the majority of the data was obtained using this strain. However, data are presented from all four of the standard tester strains. Negative controls included the top agar with no additions, the top agar with liver S9 and no additions, the top agar with 50 µL DMSO, and the top agar with liver S9 and 50 µL DMSO. Positive controls included 25 µg per plate 2-nitrofluorene for strains TA98 and TA 1538, 10µg per plate sodium azide for strains TA100 and TA1535; and 5µg benzo(a)pyrene or 10µg 2-aminoanthracene for all strains to evaluate the efficiency of the metabolic activation system. Sterility controls were also run for top agar, test chemicals, and the S9 mix for each experiment.

The data presented in Table 4-2 represent the means of the positive and solvent controls for the entire three years of the study for all of the tester strains employed in this research. The data indicate that there is a dramatic difference in the response observed in the different strains. The DMSO or solvent control values range from a low of 16 for strain TA1538 to a high of 126 for strain TA100 without activation (Table 4-2). For all strains, the solvent control values are within those defined as acceptable by de Serres and Shelby (1979).

For the control mutagen 2-aminoanthracene, the responses ranged from 187 total revertants for strain TA1535 to 1548 total revertants for strain TA98. The response induced by the polynuclear aromatic hydrocarbon benzo(a)pyrene ranged from 93 total revertants for strain TA1535 to 325 total revertants for strain TA100 (Table 4-2). For all strains, the control values for the indirect acting mutagens (2-aminoanthracene and benzo(a)pyrene) were low compared to the values reported in the literature (Ames _et_ _al_., 1975). The reduced response observed in the present study is likely due to the high level of metabolic activation employed. Although high levels of activation frequently result in lower than optimum responses for positive controls, they also have been found to be necessary for the efficient activation of complex mixtures (Donnelly _et_ _al_., 1987b). In

TABLE 4-2. POSITIVIE AND SOLVENT CONTROLS FOR S. typhimurium STRAINS EMPLOYED IN THE STUDY

Chemical (Amount)	Metabolic Activation†	Total Revertants - Means ± S.D.			
		TA98	TA1538	TA100	TA1535
None	-	29 ± 10	17 ± 4	127 ± 21	36 ± 8
	+	38 ± 16	19 ± 2	121 ± 14	17 ± 2
DMSO	-	28 ± 9	16 ± 4	126 ± 21	36 ± 11
	+	37 ± 21	22 ± 7	134 ± 30	18 ± 5
2-Nitrofluorene (25 µg)	-	1837 ± 541	1881 ± 509	-	-
Sodium Azide (10 µg)	-	-	-	1055 ± 350	1437 ± 110
2-Aminoanthracine (10 µg)	-	24 ± 8	-	249 ± 135	38 ± 6
	+	1548 ± 444	-	649 ± 197	187 ± 76
Benzo(a)pyrene (5 µg)	-	42 ± 15	17 ± 2	130 ± 75	26 ± 10
	+	278 ± 143	155 ± 34	325 ± 152	93 ± 17

† S9 mix composed of 0.3 mL rat liver per mL S9 mix.

general the response obtained for the positive controls displayed a variability of 15 to 50% over the entire three years of the project (Table 4-2).

The control values for strain TA98 for the entire project period have also been provided (Table 4-3). These data indicate that all of the solvent and positive control values were within acceptable ranges for this strain. The variability for strain TA98 with the direct acting mutagen 2-nitrofluorene was approximately 30%, while the variability for the indirect acting mutagen benzo(a)pyrene was approximately 50% (Table 4-3). Thus, the solvent and positive control values for the strains utilized in this research are within acceptable ranges.

TABLE 4-3. POSITIVE AND NEGATIVE CONTROL VALUES FOR STRAIN TA98 FOR ENTIRE PROJECT PERIOD

Control[†]	Metabolic Activation	Mean	Standard Deviation	Number of Replications
DMSO	-S9	28.26	9.23	376
DMSO	+S9	36.75	21.16	400
NONE	-S9	26.67	9.56	274
NONE	+S9	38.26	15.75	238
2-Nitrofluorene	-S9	1836.87	540.67	138
Benzo(a)pyrene	+S9	277.81	143.02	101

[†] Negative controls include NONE (2.5 mL top agar with no additions), DMSO (top agar plus 50 µL DMSO); Postivie control include 25 µg per plate 2-nitrofluorene without activation, 5 µg per plate benzo(a)pyrene with activation, and 10 µg per plate 2-aminoanthracine with activation.

These data is important to the conclusions reached when interpreting the environmental mutagenicity data as the control values are used to measure the performance and the genetic integrity of the tester strains. Abnormally high or low negative control values usually indicate a loss of genetic integrity and Ames et al. (1975) suggest that if this occurs the strains should be reisolated. Failure to obtain a positive response with the standard mutagens similarly indicates a misfunction of the tester strain or the media preparations.

All of the data obtained from the environmental samples collected for this study was obtained using strains which had been adequately tested and induced the anticipated response in the concurrent solvent or positive control. In addition, since the variability of the strains appears to be at least 30% between independent experiments, it should be assumed that differences of less than 30% for the various environmental samples have no toxicological significance.

SECTION 5

SLUDGE CHARACTERIZATION

The composition of sludge from a municipal wastewater treatment plant will likely depend on the quantity and quality of industrial and domestic contributions. A partial listing of organic constituents reported in the literature as being present in municipal sewage (Section 1, Table 1-1) indicates that a variety of chemicals may be present in sludge including chlorinated hydrocarbons and polynuclear aromatic hydrocarbons. While the genotoxic potential of most of the chemicals in Table 1-1 has not been well characterized, many are established carcinogens. Most of these organic compounds are assumed to be representative of the industrial contribution to municipal sewage.

There is also reason to believe that certain compounds of domestic origin may have a negative impact on the quality of municipal sewage. Mutagenic activity has been detected in a variety of foodstuffs including fish (Marquardt et al., 1977), hamburger (Commoner et al., 1978; Felton et al., 1981; Rhee et al., 1987), broiled foods (Sugimura et al., 1977), vegetables (van der Hoeven et al., 1982), coffee (Aeschbacker and Wurnzer, 1980) and beer (Knudsen et al., 1979). While it is not known if these mutagens are passed through to the sewage, mutagenic activity has been detected in the feces of a population at a high risk for colon cancer (Ehrich et al., 1979), and in the urine of cigarette smokers (Yamasaki and Ames, 1977), as well as the urine of workers in the rubber industry (Falck et al., 1980).

Nitrosamines represent another carcinogenic contaminant which may be of domestic origin in sewage sludge (Green et al.,1981). Ayanaba et al. (1973) demonstrated that microorganisms could generate nitrosamines from their precursors in sewage. In addition, Tate and Alexander (1975) observed that microbial decomposition of nitrosamines in soil and sewage was very slow. Kearney et al. (1980) however, stated that land application of trace amounts of nitrosamines presents no threat to the food chain because nitrosamines will degrade rapidly in a soil environment.

Although municipal sludges are not likely to contain high concentrations of any specific contaminant, previous research has indicated that the solvent extract of sludge may induce a strong mutagenic response in bacterial assays (Hopke et al., 1984; Tabor et al., 1985; Donnelly et al., 1987a). This research was conducted to evaluate the bacterial mutagenicity of municipal sludge from a variety of different sources.

MATERIALS AND METHODS

Municipal Sludge Selection

A total of seventeen municipal sewage sludge samples were collected, extracted, and tested in the Salmonella/microsome assay. Eleven sludge samples were collected in Texas, two samples were collected in Illinois, and four samples were collected in Washington by personnel from Battelle, NW. A brief summary of the sludge characteristics is provided in Table 5-1.

TABLE 5-1. GROSS CHARACTERISTICS OF SEWAGE SLUDGE SAMPLES

Source†	Date Collected	% Industrial	% Water	% Ash	Solvent Extractable Organics (mg/g)		
					CH_2Cl_2	CH_3OH	Total
SMO	12/27/84	19	87	5.7	21.3	0.03	21.3
SM1	1/25/85	19	10	36.0	40.9	34.7	75.6
SM2	2/22/85	19	15	-	49.4	53.0	102.4
SM3	12/9/85	19	9	-	5.3	3.9	9.2
SM4	5/8/86	19	11	-	6.3	6.3	12.6
SM5	8/4/86	19	10	-	2.4	1.8	4.2
SM6	10/1/86	19	9	-	5.6	3.0	8.6
JT1	1/25/85	6	87	5.5	13.0	32.3	45.3
JT2	2/22/85	6	16	37.0	54.5	36.5	91.1
VG	1/31/85	20	30	64.0	5.1	1.3	6.4
BN	2/14/85	50	68	24.0	25.8	12.2	38.0
RD	3/27/85	?	72	14.0	26.4	6.7	33.1
ST	4/17/85	?	78	15.0	65.3	38.2	103.5
TA	4/17/85	?	85	10.4	17.4	6.7	24.1
SK	4/17/85	?	82	10.2	54.4	32.2	86.6
AD1	9/15/86	?	-	-	2.9	2.3	5.2
AD2	10/11/86	?	-	-	4.5	2.5	7.0

† Sources described in more detail in text. Numbers denote sample collected from same site on different dates or different points in treatment process.

For convenience, each sample was given a two letter identification code; multiple samples collected from a single source are followed by a number. Sludges collected in Texas include SM0, SM1, SM2, SM3, SM4, SM5, SM6, JT1, JT2, VG, and BN. Sludges collected in Washington include ST, TA, SK, and RD. Sludges collected in Illinois included AD1 and AD2.

A total of nine sludge samples were collected from three different sewage treatment plants in a large south Texas city. Mr. Lonnie Lange and Mr. Dennis Gallagher provided assistance in the selection and collection of samples. The first sample (SM0), collected in December of 1984, was a wet sludge (86% water) from the Eimco* vacuum filter at a wastewater treatment plant in a highly industrialized area. Shortly after this sample was collected, this plant was closed for renovations, and the influent to this plant was pumped to a second plant (SM). In January 1985, two samples were collected from this city. One sample was a dried sludge (10% water) from the flash drier at a plant similar to the one sampled in December (SM1), and the second was a wet sludge from the Eimco vacuum filter at a different plant in a region of the city including both domestic and industrial areas (JT1). Two additional samples from this city were collected in February, 1985. These included one sample from the wastewater treatment plant located in a heavily industrialized area (SM2), and one from the mixed domestic and industrial plant sampled in January (JT2). In December of 1985, another sample was collected from the SM plant (SM3). The final three sludge samples from the south Texas city were collected in May, August and October from the industrialized plant sampled in February

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*Mention of brand names do not constitute endorsement.

(SM4, SM5, SM6). All of these samples were dried sludges collected from the C.E. Raymond cage mill flash drier.

The treatment process is essentially the same at all three plants. The wastewater first undergoes re-aeration. The wastewater is then pumped to a constant head holding tank. From the holding tank, $FeCl_3$ is added, and the wastewater is pumped to an Eimco vacuum filter where the moisture content of the sludge is reduced to approximately 85%. The final step in the treatment process is to pump the sludge to a C.E. Raymond cage mill flash dryer where the sludge moisture content is reduced to approximately 15% using heat and vacuum.

An attempt was made to select the three treatment plants in south Texas to represent a range of industrial contribution. Although the selected plants were in areas that were either predominantly domestic or industrial, the overall contribution to each plant was approximately equal. The first plant treats approximately 22 million gallons of sewage each day. The predominant industries in the vicinity of the plant are manufacturing. Approximately 19% of the waste treated at the plant is of industrial origin. The second plant is located in a mixed domestic and industrial area. This plant treats approximately 88 million gallons of sewage per day of which 6% is of industrial origin. Industries contributing to this plant include food processing, brewing and manufacturing. The third plant serves a population of almost 300,000 and treats approximately 38 million gallons of wastewater each day, including approximately 20% from industries. The primary industries contributing to this plant are manufacturing.

One sludge sample was collected from a wastewater treatment plant in a large north Texas city (VG). Mr. Robert McMillon assisted in the collection and selection of the sludge sample. This plant serves approximately 650,000 people and treats an average of 110 million gallons of municipal wastewater per day. Approximately 20% of this sewage is generated by industries, i.e., primarily brewing and food processing with smaller contributions from dairy, paint and machine shop industries. Two types of sludge are generated at this plant. Approximately 40% of the sludge is a primary sludge, while 60% is a waste activated sludge. Sludge treatment includes a primary settling tank, a secondary settling tank, and an anaerobic digester where the sludge is heated and mixed for 20 to 25 days. The sludge is pumped from the anaerobic digester to large drying beds where the solids settle and the aqueous phase is periodically decanted. When the drying beds are full, the sludge is removed to a large storage area where it is retained for application to parks and highway right of ways. The dried sludge pile was the source of the sample used in this study.

One sludge sample was collected at a wastewater treatment plant in a medium sized central Texas city (BN). Mr. David Wallet provided assistance in the selection and collection of sludge samples. The central Texas plant treats approximately 2 million gallons of sewage per day. Primarily oil related industries contributed almost 50% of the sewage treated by this plant. Sewage treated at this plant is first passed through a grit remover, then to a primary clarifer, followed by a trickling filter, a final clarifier, and an anaerobic digester. Sludge from the anaerobic digester is pumped to drying beds where the sludge is dried prior to removal to a landfill. The dried sludge was used in the present study.

This sludge had a moisture content of 68% and a solvent extractable organic content of 38 mg/g.

Two sludge samples were collected, one each in September and October from a wastewater treatment plant in Illinois (AD1, AD2). These samples were collected from drying beds, and had a moisture content of 63%. The remaining four sludge samples were collected from cities in Washington state by Batelle, NW and were shipped to us by Dr. Walter Weimer. These included two gallons each of sludges from four different cities in Washington (ST, TA, SK, and RD). The sludge moisture contents were 85% for TA, 82% for SK, 78% for SK, and 72% for RD. Total solvent extractable organics were 33.1, 103.5, 24.1 and 86.6 mg/g for RD, ST, TA, and SK, respectively. No details were provided to describe the treatment process or industrial contributions to these sludges.

Extraction

The sludge samples were sequentially extracted with CH_2Cl_2 and CH_3OH using a Soxhlet apparatus or a Tecator Soxtec apparatus (Tecator, Hoganas, Sweden). In order to evaluate the influence of pentane on the recovery of mutagens from the sludges, one sludge sample (SM3) was sequentially extracted using pentane, CH_2Cl_2, and CH_3OH. Sludge samples were dried at 60° for 24hr prior to extraction. The loss of semi-volatile compounds during the drying process was not considered a significant problem as the protocol for the extraction requires that the sample be extracted at appreciably greater temperatures. For the initial eleven sludge samples, fifteen to twenty grams of dried sludge was combined with an equal weight of anhydrous sodium sulfate and weighed into a cellulose extraction thimble and mixed with a glass rod. The thimble and glass rod were placed in a 200 mL capacity Soxhlet extractor. Condensors and boiling flasks containing 200 mL of solvent were assembled with the extractor and placed on the heater apparatus. Refrigerated coolant was circulated through the condensers. A 30 mL volume of solvent was introduced into the extractor chamber. Heat was applied to the boiling flask to initiate a 6hr solvent extraction of the organic components; the temperature was adjusted to give six flushings per hour.

The fourth, fifth, sixth and seventh samples collected from the SM wastewater treatment plant were extracted using the Tecator Soxtec apparatus. Fifteen to twenty gram samples were weighed and added to the cellulose extraction thimbles following the same procedures as used for the Soxhlet apparatus. The thimbles were then placed in the thimble support and attached to the thimble holder. The glass cups were then filled with 25 mL of CH_2Cl_2 or CH_3OH and the cups clamped into the condensors. Each sample was extracted for 5 min in the boiling position and 55 min in the rinsing position. After the rinsing time was completed, the condensor valves were closed and the residual solvent evaporated to dryness. All extractions were conducted in triplicate and composited. Two fractions were thus sequentially collected from each dried sample, a CH_2Cl_2 and a CH_3OH fraction. The residue from each extraction was then weighed, redissolved in dimethyl sulfoxide (DMSO-Grade I, Sigma Chem. Co., St. Louis, MO), and an aliquot of each fraction combined in equal portions to represent a third composite fraction.

Bioassay

The *Salmonella*/microsome assay of Ames *et al.* (1975) was used to evaluate the mutagenicity of the solvent extractable organic compounds in the sludge fractions. The Salmonella strains were kindly supplied by Dr. B.N. Ames (University of California, Berkeley, CA). The procedural methods were the same as Ames *et al.* (1975) with the modifications suggested by Maron and Ames (1983). The Salmonella tester strain TA98 was utilized with and without metabolic activation. Selected samples were also tested with strains TA100, TA1538 and TA1535. The S9 mixture (9,000 x g supernatent from homogenized liver) contained 0.3 mL rat-liver S9 and 0.7 mL cofactor supplement (11.4 mM $MgCl_2$, 47 mM KCl, 7.1 mM glucose-6-phosphate, 5.7 mM NADP and 140 mM potassium phosphate buffer at pH 7.4). Aroclor induced Sprague-Dawley rat liver was obtained from Organon Teknika (West Chester, PA). The addition of mammalian metabolism to the assay system is designed to mimic the metabolism which would occur in an exposed mammalian population, and allows the detection of both direct and indirect acting mutagens. Chemicals which do not require metabolic activation to reach their ultimate mutagenic form are described as direct acting, while those which must be metabolized to reach their mutagenic form are described as indirect acting. One sludge fraction was tested at three different levels of metabolic activation in order to determine the optimum level of activation for the types of sludge samples tested. The three levels employed included 0.04 mL rat liver per mL S9 mix (low), 0.1 mL rat liver per mL S9 mix (medium), and 0.3 mL rat liver per mL S9 mix (high).

Each of the three fractions obtained from the sludge was tested in the standard plate incorporation assay at a minimum of five dose levels (mg/plate): 0.5, 1.0, 2.5, 5.0, and 10.0. Bioassays were conducted on a total of 36 fractions from 12 municipal sewage sludges. Five additional sludge samples (AD1, AD2, SM4, SM5, SM6) were collected and extracted later in the study including three which were repeat samples from the SM treatment plant. All of the sludge fractions were tested in strain TA98 and six fractions were tested in both strain TA98 and TA100, while three of these fractions from the SM3 sludge were also tested in strains TA1535 and TA1538. The data were analyzed using the modified two-fold rule (Chu et al., 1981). Using this procedure, a response is considered positive if the average response for at least two consecutive dose levels was greater than twice the average response for the concurrent negative/solvent control (Chu et al., 1981).

All strains were checked monthly for nutritional markers (histidine and biotin) and other genetic characteristics, i.e., sensitivity to crystal violet, ampicillin, and UV light. On each test date, all tester strains were calibrated with positive and negative controls. Positive controls included 25 μg/plate 2-nitrofluorene (Aldrich, Milwakee, WI) for TA98 and TA1538, and 1 μg/plate sodium azide (Sigma, St. Louis, MO) for TA100 and TA1535. Benzo[a]pyrene (Sigma) at 5 μg/plate was used with all strains to verify the functioning of the metabolic activation system. All reagents and extracts were tested for sterility; dimethyl sulfoxide (DMSO, Grade I, Sigma) was used as a solvent control.

RESULTS AND DISCUSSION

The gross characteristics of the sludge samples are provided in Table

5-1. The sludge samples collected with relatively high moisture contents (greater than 68 per cent by weight) included SM0, JT1, BN, ST, TA, SK, and RD. The sludge samples with low moisture contents (less than 30 per cent by weight) included SM1, VG, SM2, JT2, and SM3, 4, and 5. Ash content of the solid phase of the sludge ranged from a low of 5.5% for the JT1 sludge, to a high of 64% for the VG sludge. The total solvent extractable organic matter of the solid phase of the sludge ranged from a low of 4.2 mg/g for the SM5 sludge to a high of 102 mg/g for the SM2 sludge. These data indicate that the total and extractable organic carbon content of municipal sludge may vary greatly between different sources.

None of the fractions from the SM3 sludge induced a positive response in strain TA100 or TA1535 with or without metabolic activation (Table 5-2). A slight but less than two-fold increase in the total revertant colonies was observed with the CH_3OH fraction with strain TA100 (Table 5-2). The same effect was not observed in strain TA1535. Although strain TA98 was more sensitive to mutagens in the methanol fraction, most of the sludge samples which induced a response in strain TA98 also induced a comparable response in strain TA1538 (Table 5-2). The relative response of the CH_3OH fraction with metabolic activation in strain TA98 was approximately twice that observed for strain TA1538 (Table 5-2). For the remaining sludge fractions, the data will be discussed only in terms of strain TA98.

The results (Table 5-3 and Figure 5-1) indicate that the use of pentane in addition to CH_2Cl_2 and CH_3OH for solvent extraction of the sludge did not affect the mutagenic potential of the sludge fractions. Using sequential soxhlet extraction, the pentane, CH_2Cl_2, CH_3OH and

TABLE 5-2. MUTAGENIC POTENTIAL OF SOLVENT EXTRACTABLE ORGANIC COMPOUNDS FROM SM3 SEWAGE SLUDGE AND DATA FROM CONTROLS

Fraction	Dose (mg) plate)	Total his$^+$ revertants (Mean ± SD)							
		Strain: TA 98		TA1538		TA100		TA1535	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Methylene Chloride	0	32 ± 2	41 ± 3	16 ± 1	27 ± 6	128 ± 8	131 ± 12	34 ± 4	17 ± 4
	0.5	34 ± 12	39 ± 7	17 ± 1	17 ± 3	120 ± 7	122 ± 15	31 ± 7	19 ± 7
	1.0	44 ± 9	40 ± 11	37 ± 18	30 ± 8	94 ± 12	115 ± 11	22 ± 6	24 ± 8
	2.5	75 ± 6	52 ± 8	85 ± 9	37 ± 12	105 ± 17	118 ± 23	16 ± 5	17 ± 3
	5.0	184 ± 17	49 ± 8	101 ± 11	35 ± 4	112 ± 6	146 ± 25	13 ± 7	14 ± 6
	10.0	151 ± 14	66 ± 13	104 ± 25	63 ± 8	140 ± 31	174 ± 21	21 ± 3	16 ± 9
Methanol	0	33 ± 5	48 ± 7	16 ± 1	27 ± 6	128 ± 8	131 ± 12	34 ± 4	17 ± 4
	0.5	33 ± 3	52 ± 8	14 ± 9	15 ± 5	116 ± 10	125 ± 20	27 ± 8	16 ± 3
	1.0	34 ± 3	63 ± 9	15 ± 7	19 ± 3	120 ± 18	119 ± 11	25 ± 8	15 ± 2
	2.5	36 ± 5	97 ± 29	59 ± 38	46 ± 3	126 ± 10	115 ± 18	26 ± 13	16 ± 6
	5.0	43 ± 11	126 ± 48	36 ± 11	54 ± 4	121 ± 26	101 ± 11	28 ± 6	15 ± 5
	10.0	35 ± 3	135 ± 15	41 ± 18	69 ± 5	109 ± 21	140 ± 11	26 ± 8	14 ± 4
Combined	0	27 ± 5	43 ± 12	16 ± 1	27 ± 6	128 ± 8	131 ± 12	34 ± 4	17 ± 4
	0.5	35 ± 13	57 ± 7	9 ± 6	19 ± 6	131 ± 16	121 ± 22	27 ± 2	15 ± 5
	1.0	45 ± 20	81 ± 17	30 ± 7	20 ± 6	114 ± 13	109 ± 26	24 ± 4	14 ± 1
	2.5	60 ± 16	78 ± 27	42 ± 23	20 ± 5	123 ± 18	107 ± 9	21 ± 7	17 ± 3
	5.0	110 ± 21	86 ± 16	41 ± 17	33 ± 17	108 ± 8	112 ± 18	32 ± 8	14 ± 3
	10.0	107 ± 13	114 ± 26	110 ± 14	46 ± 17	119 ± 12	118 ± 17	26 ± 3	16 ± 6
None	-	29 ± 10	38 ± 16	17 ± 4	19 ± 2	127 ± 21	121 ± 14	36 ± 8	16 ± 2
DMSO	50µl	37 ± 21	16 ± 4	37 ± 21	22 ± 7	126 ± 21	134 ± 30	36 ± 11	18 ± 5
2-Nitro-fluorene	0.025	1837 ± 541	NT	1881 ± 509	NT	NT	NT	NT	NT
Sodium Azide	0.001	NT	NT	NT	NT	1055 ± 350	NT	1437 ±110	NT
Benzo(a) pyrene	0.005	34 ± 11	278 ± 143	17 ± 2	155 ± 34	130 ± 75	325 ± 152	38 ± 6	187 ± 76
2-Amino-anthracine	0.010	29 ± 13	954 ± 544	NT	NT	249 ± 135	649 ± 197	26 ± 10	63 ± 3

NT = not tested.

TABLE 5-3. MUTAGENICITY OF SOLVENT EXTRACTABLE ORGANIC COMPOUNDS FROM SM2 SLUDGE EXTRACTED WITH (SM2A) AND WITHOUT (SM2B) PENTANE AS THE PRIMARY EXTRACTANT

		EXTRACTANT							
		Pentane		CH_2Cl_2		CH_3OH		Combined	
Sample	Dose (mg) plate)	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
		Total TA98 his^+ Revertants (Mean ± SD)							
SM2A	0.0	36 ± 3.9	32 ± 7.7	36 ± 3.9	32 ± 4.7	36 ± 3.9	32 ± 4.7	36 ± 7.2	32 ± 4.7
	0.5	22 ± 3.0	25 ± 6.3	36 ± 10.0	24 ± 3.8	22 ± 3.4	35 ± 2.4	19 ± 6.1	29 ± 12.0
	1.0	27 ± 4.3	26 ± 9.9	42 ± 15.0	29 ± 4.4	18 ± 2.8	44 ± 10.0	31 ± 8.2	54 ± 17.0
	2.5	43 ± 14.0	24 ± 2.9	45 ± 11.0	45 ± 6.4	27 ± 8.0	36 ± 7.0	40 ± 4.7	51 ± 4.6
	5.0	50 ± 14.0	27 ± 9.4	57 ± 18.0	59 ± 5.3	36 ± 5.2	121 ± 36.0	52 ± 18.0	67 ± 12.0
	10.0	69 ± 8.7	39 ± 21.0	59 ± 19.0	109 ± 11.0	39 ± 11.0	53 ± 9.5	61 ± 8.8	63 ± 9.9
SM2B	0.0	NT	NT	32 ± 4.5	29 ± 9.4	32 ± 5.3	37 ± 3.7	32 ± 3.8	31 ± 12.0
	0.1	NT	NT	23 ± 3.6	31 ± 4.5	NT	NT	21 ± 8.8	24 ± 8.2
	0.5	NT	NT	24 ± 5.7	31 ± 8.1	30 ± 4.1	54 ± 20.0	20 ± 4.6	32 ± 5.7
	1.0	NT	NT	26 ± 11.0	38 ± 10.0	30 ± 12.0	58 ± 26.0	20 ± 6.8	37 ± 3.2
	2.5	NT	NT	NT	NT	31 ± 5.7	89 ± 54.0	NT	NT
	5.0	NT	NT	42 ± 24.0	47 ± 21.0	36 ± 6.0	115 ± 32.0	29 ± 10.0	33 ± 13.0
	10.0	NT	NT	43 ± 49.0	119 ± 11.0	49 ± 12.0	71 ± 45.0	41 ± 5.0	36 ± 10.0

NT = not tested.

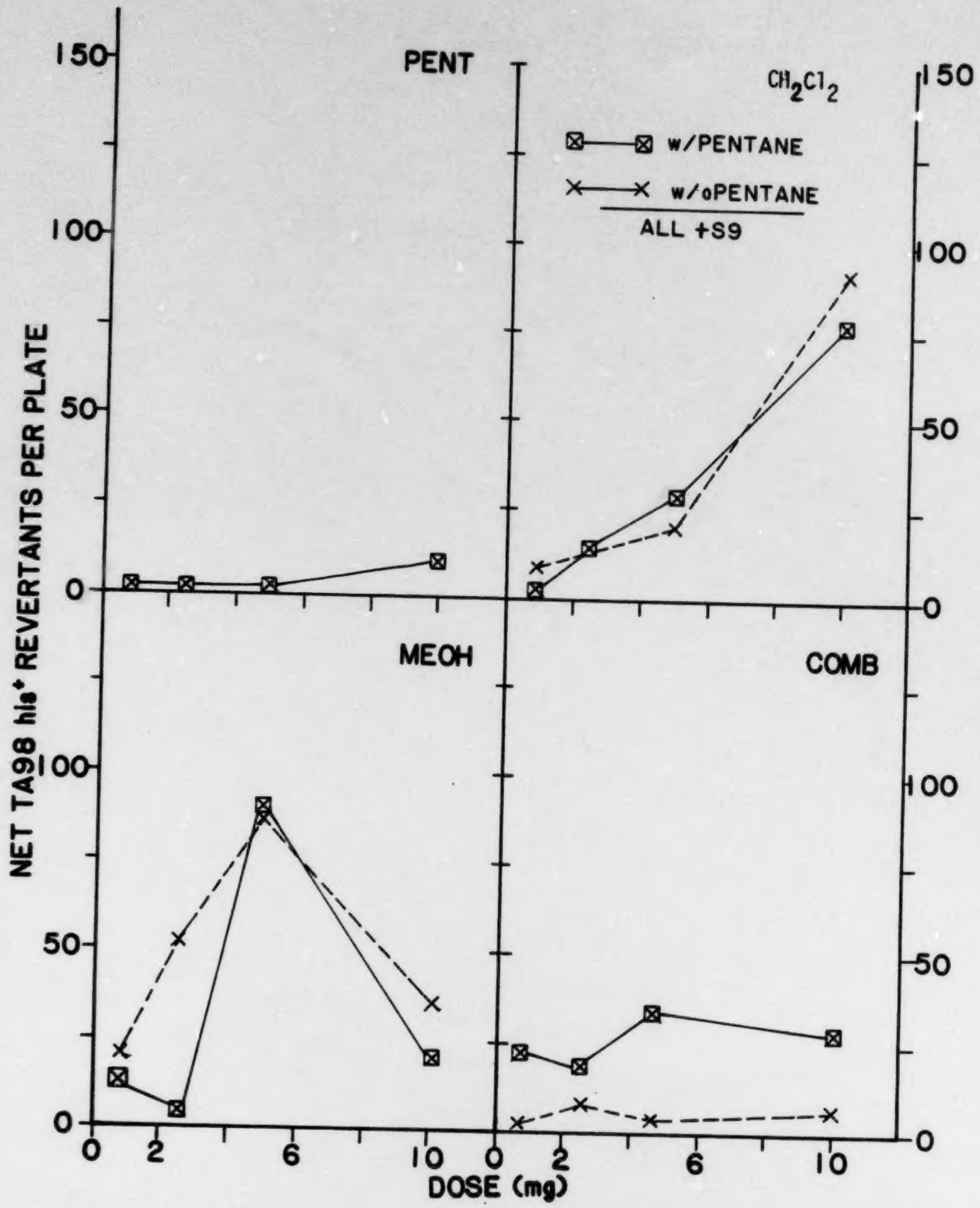


Figure 5-1. Mutagenic potential of SM2 sludge extracted sequentially with pentane (PENT), methylene chloride (CH_2Cl_2), and methanol (CH_3OH) or methylene chloride and methanol (COMB).

combined fraction induced responses that were 1.2, 3.4, 3.8, and 2.1 times the solvent control, respectively, at the optimum dose level with metabolic activation. When the same sludge sample was extracted using only CH_2Cl_2 and CH_3OH, the mutagenic responses were 4.2, 3.3, and 1.2 times background for the CH_2Cl_2, CH_3OH, and combined fractions, respectively (Figure 5-1 and Table 5-3). Thus, the use of pentane as the initial extracting solvent appeared to have little influence on the mutagenic potential of the CH_2Cl_2 or CH_3OH fraction, although the mutagenicity of the combined fraction increased slightly when pentane was added to the extraction protocol.

A study was conducted with the SM3 sludge using either the Soxhlet procedure, or one of three procedures using the Tecator apparatus to determine the optimum extraction procedure for the sludge samples to be used in this project. The results (Table 5-4) indicate that there was no appreciable difference in the quantity of organics obtained in the CH_2Cl_2 fraction using any of the four procedures. However, while the CH_3OH fractions extracted using the three variations of the Tecator procedure were approximately equal, the quantity of organics in the CH_3 fraction extracted using the Soxhlet procedure was almost double that obtained using the Tecator (Table 5-4).

The results from the biological analysis of the sludge fractions obtained using the four different extraction procedures are presented in Table 5-5, Figures 5-2 and 5-3. There was no appreciable difference in the bacterial mutagenicity of the CH_2Cl_2 fraction obtained using either the Soxhlet or Tecator 5/55 (extraction performed using 5 min boil and 55 min rinse) extraction procedure, while no mutagenic activity was observed in

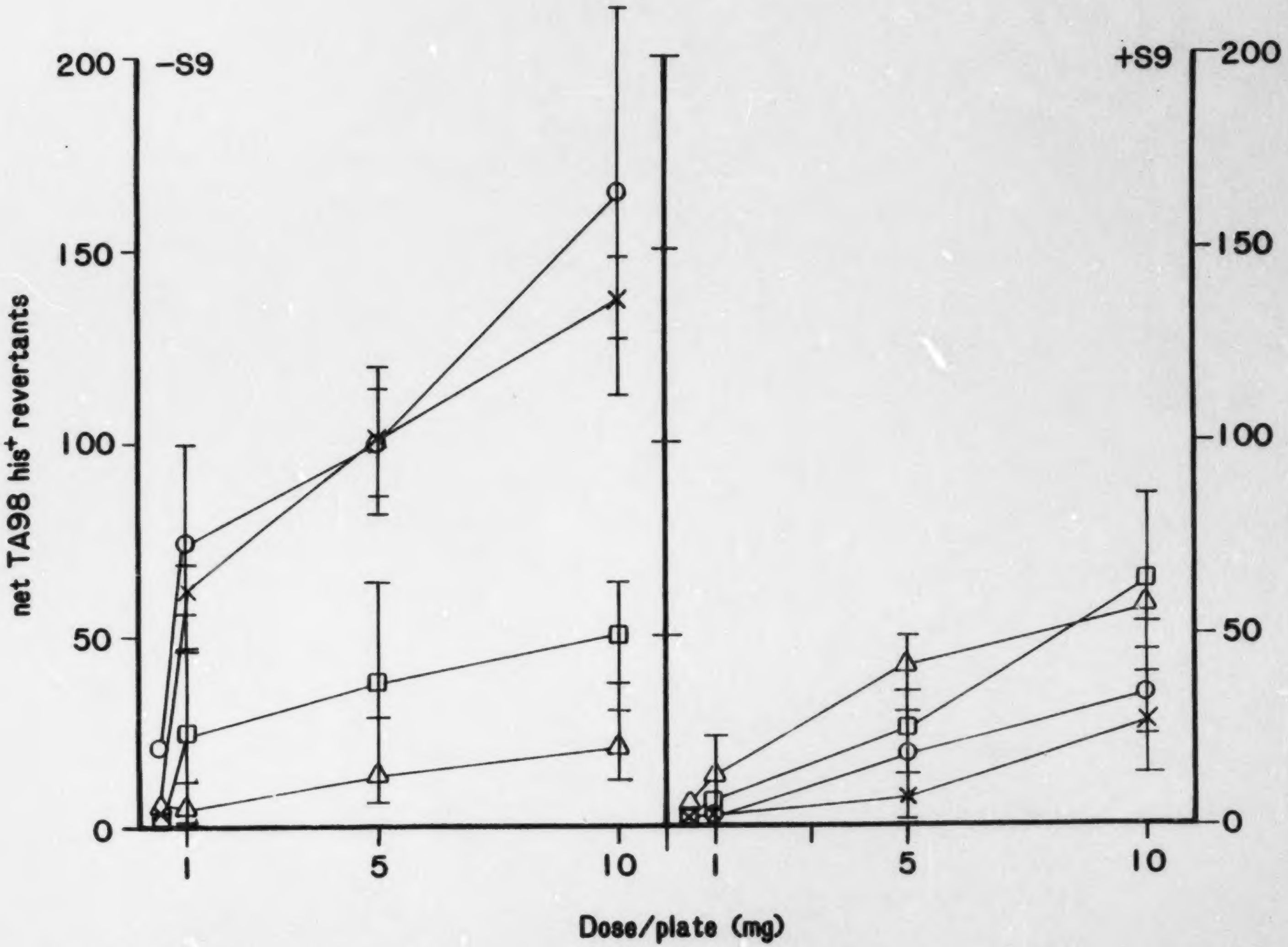


Figure 5-2. Bacterial mutagenicity of methylene chloride fraction obtained using standard Soxhlet (X), or Tecator extraction with 5 min boil, 55 min rinse (O), 5 min boil, 115 min (Δ), or 10 min boil, 50 min rinse (□).

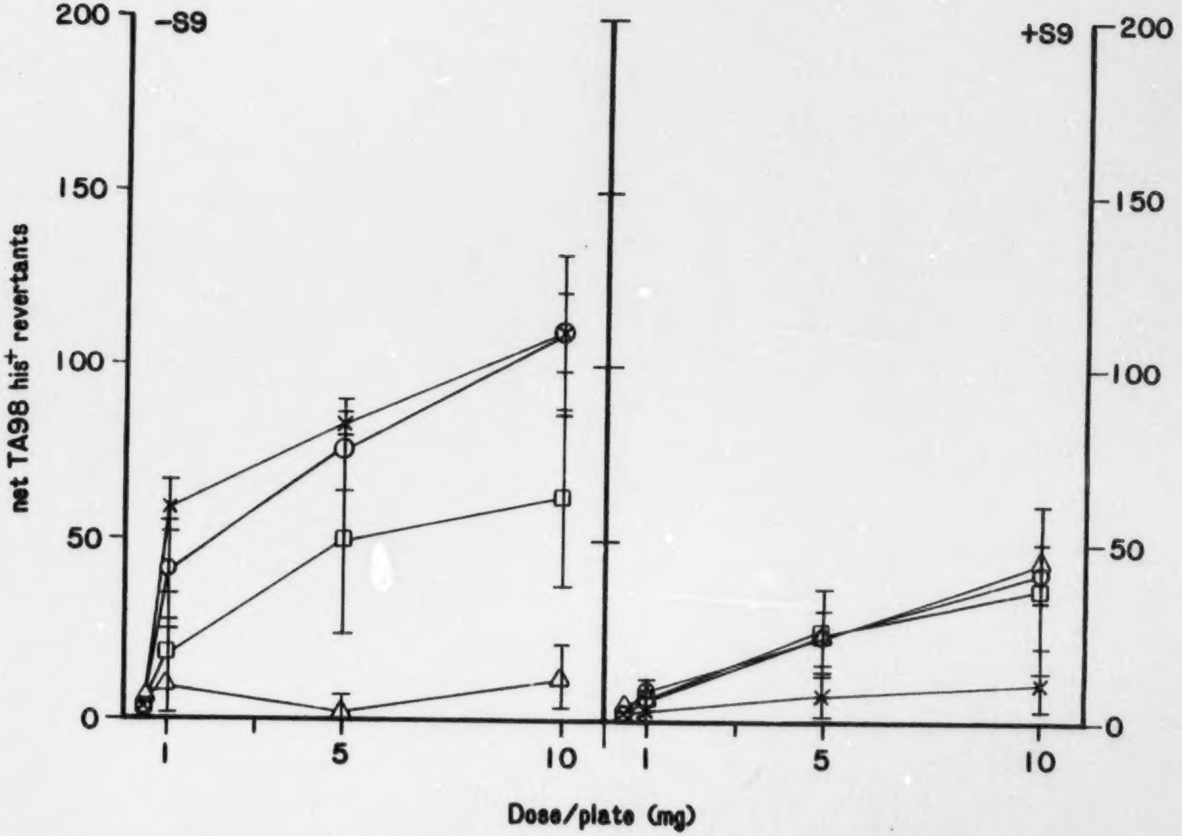


Figure 5-3. Bacterial mutagenicity of combined methylene chloride and methanol fraction obtained using standard Soxhlet (X), or Tecator extraction with 5 min boil, 55 min rinse (O), 5 min boil, 115 min (Δ), or 10 min boil, 50 min rinse (□).

TABLE 5-4. SOLVENT EXTRACTABLE ORGANIC COMPOUNDS RECOVERED FROM THE SM3 SLUDGE SAMPLES SUBJECTED TO FOUR DIFFERENT EXTRACTION PROCEDURES. (ALL EXTRACTIONS CONDUCTED IN TRIPLICATE).

Procedure †	Solvent Extractable Organics (mg/g) ††		
	CH_2Cl_2	CH_3OH	Total
Tecator 5/55	63 ± 6.6	29 ± 14.0	92 ± 18.0
Tecator 5/115	65 ± 6.1	39 ± 5.0	104 ± 9.3
Tecator 10/50	59 ± 2.6	30 ± 6.5	89 ± 2.9
Soxhlet	67 ± 9.0	63 ± 17.0	130 ± 25.0

† Extraction procedure: for Tecator extractions first number equals boiling time, second number equals rinse time; for Soxhlet, extraction was conducted continuously for six hours.

†† Values represent Mean ± S.D; CH_2Cl_2 = methylene chloride extractable; CH_3OH = methanol extractable; Total = total solvent extractable organics.

the CH_2Cl_2 fraction obtained using either the Tecator 10/50 or 5/115 extraction procedure (Table 5-5 and Figure 5-2). For the CH_3OH fraction, none of the extraction procedures yielded a residue which induced a positive response in the bioassay (Table 5-4). For the combined fractin, the residue obtained using the Soxhlet and Tecator 5/55 procedures again yielded equivalent positive responses, while the residue from the other procedures yielded a weak or negative response (Table 5-5 and Figure 5-3). Since the procedure recommended by the EPA (1985) for the preparation of soil and sediment samples for mutagenicity testing is the Soxhlet

TABLE 5-5. MUTAGENIC POTENTIAL OF SM3 SLUDGE FRACTIONS EXTRACTED USING STANDARD SOXHLET PROCEDURE OR THREE VARIATIONS OF TECATOR PROCEDURE

Procedure[†]	Dose/Plate (mg)	Net TA98 His^+ Revertants (mg/g)[††]	
		CH_2Cl_2	Combined
Soxhlet	10	136 ± 19.0	109 ± 25.0
	5	104 ± 11.0	84 ± 5.3
	1	62 ± 4.5	58 ± 4.5
Tecator 5/55	10	169 ± 56.0	109 ± 11.0
	5	103 ± 19.0	75 ± 13.0
	1	74 ± 27.0	41 ± 14.0
Tecator 5/115	10	17 ± 17.0	11 ± 7.0
	5	14 ± 17.0	3 ± 3.0
	1	5 ± 7.0	13 ± 18.0
Tecator 5/115	10	50 ± 18.0	61 ± 32.0
	5	38 ± 28.0	52 ± 28.0
	1	25 ± 22.0	17 ± 16.0

[†] Extraction procedure: Soxhlet - standard extraction procedure conducted continuously for six hours. Tecator - first number equals boiling time, second number equals rinse time.

[††] Values represent Mean ± S.D (N=12) of three replicate treatments tested on duplicate plates in two independent experiments obtained in the absence of metabolic activation. CH_2Cl_2 = methylene chloride extractable organics; Combined = combined methylene chloride and methanol extractable organics.

extraction, the Soxhlet procedure will be assumed to provide adequate extraction of bacterial mutagenicity. Thus, for the purposes of this study it will be assumed that the Soxhlet and Tecator 5/55 procedures provide approximately equal recovery of organic mutagens from the sludge or sludge amended soil.

The reproducability of the mutagenic potential in solvent extracts of one of the SM sludges prepared using either the Soxhlet, or the Tecator extraction procedure is provided in Figure 5-3. The data indicate that the Soxhlet procedure provided extremely reproducible results. At the three highest dose levels tested, the mutagenic response observed in the CH_2Cl_2 fraction extracted using the Soxhlet procedure varied by approximately 10 per cent. For the fractions extracted using the Tecator extraction procedure, a larger variance was observed (Figure 5-4). At a dose of 10 mg per plate, the response induced by the CH_2Cl_2 fraction varied by more than 50%. However, for both procedures, the optimum (dose at which the maximum mutagenic response was observed), and no-effect (highest dose at which doubling of revertants is not observed) dose level were the same. Thus, the two procedures appeared to provide reproducible results within the normal variance of the bioassay procedure.

To optimize the metabolic activation of sludge mutagens, a separate experiment was conducted using the CH_3OH fraction of the AD2 sludge. The results presented in Table 5-6 and Figure 5-5 indicate that the maximum mutagenic response was observed at the highest level of metabolic activation (0.3 mL S9/ mL S9 mix). At the lowest level of activation (0.04 mL S9/mL S9 mix), a mutagenic response was not observed at any of the dose levels tested. The maximum response observed with the medium level of activation (0.1 mL S9/mL S9 mix) was 60 net revertants at a dose of 5 mg per plate, and 99 net revertants at a dose of 10 mg per plate (Table 5-6). At the highest level of metabolic activation tested (0.3mL S9/ mL S9 mix), the CH_3OH fraction induced 72 and 130 net revertants at a dose of 5 and 10 mg per plate, respectively. The results of this independent

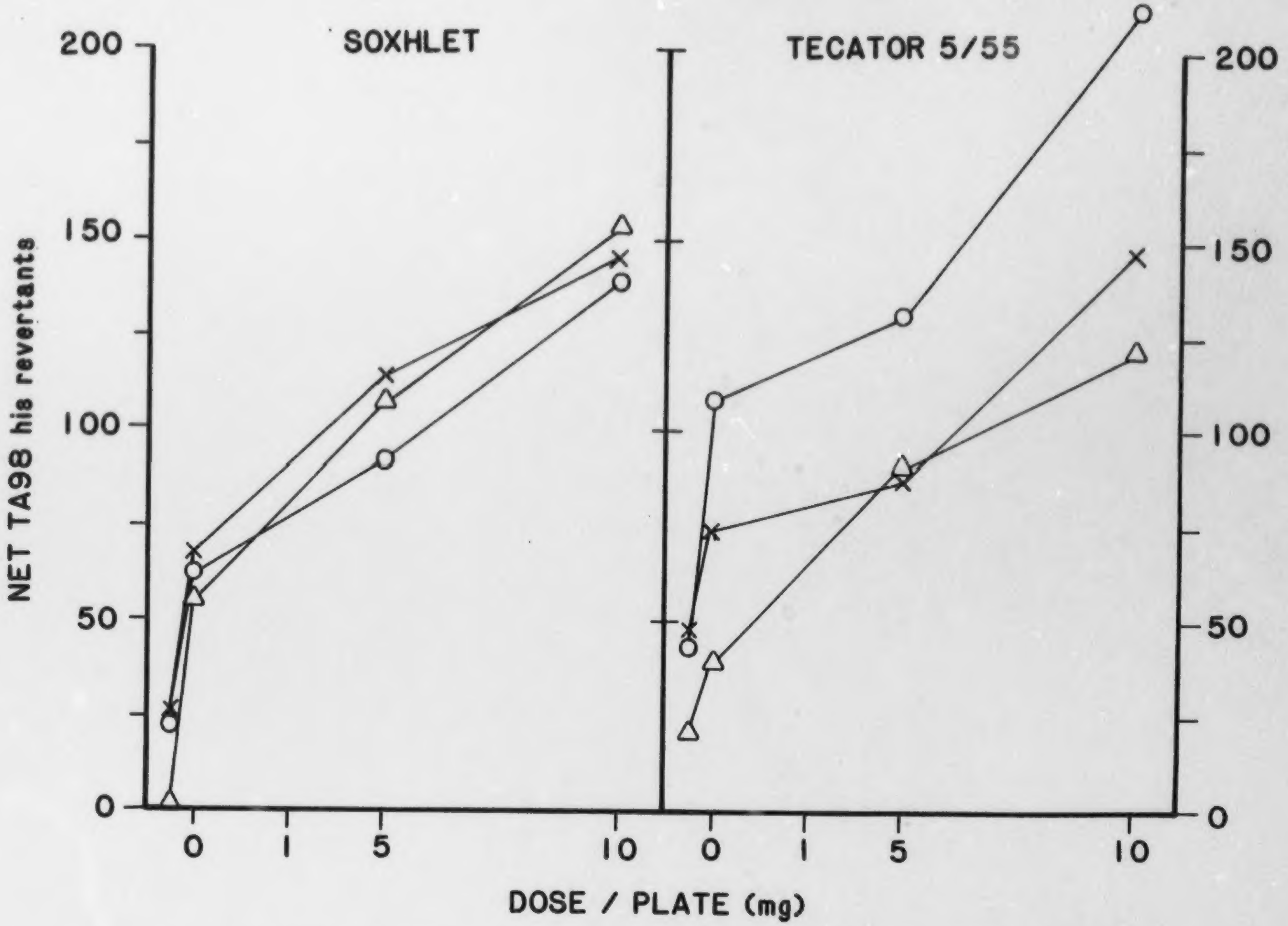


Figure 5-4. Bacterial mutagenicity of three replicate extractions of SM sludge using Soxhlet or Tecator extractor.

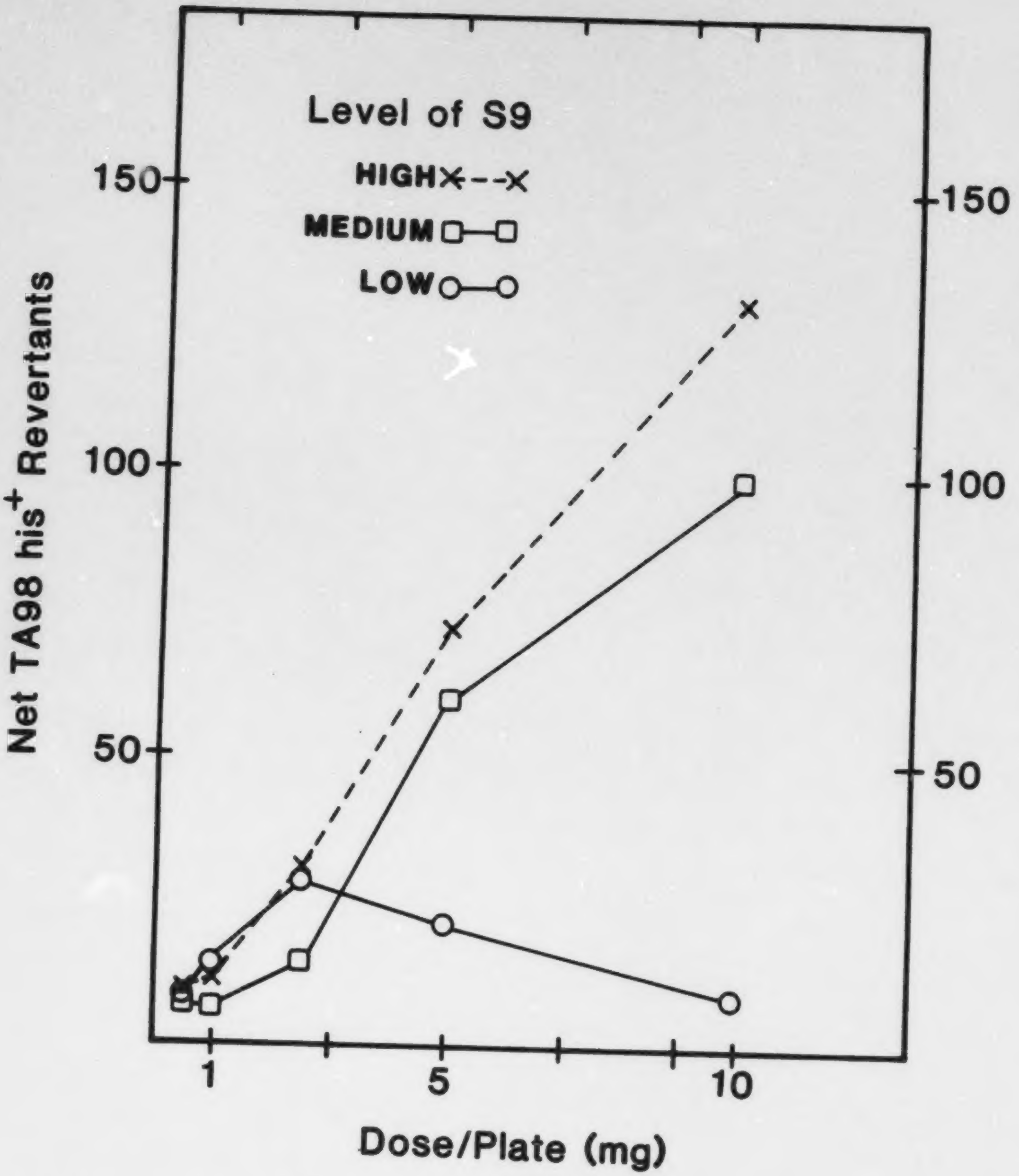


Figure 5-5. Mutagenic potential of AD2 sludge as measured using low (0.04 mL S9/mL S9 mix), medium (0.10 mL S9/mL S9 mix), or high (0.30 mL S9/mL S9 mix) level of metabolic activation.

experiment indicate that for the CH_3OH fraction of the AD2 sludge, the optimum level of microsome preparation was 0.3 mL/mL of S9 mix. Thus, the highest level of metabolic activation was determined to be the optimum and was selected for testing the sludge amended soil and leachate samples (Section 6).

TABLE 5-6. BACTERIAL MUTAGENICITY OF THE METHANOL FRACTION OF AD2 SLUDGE AS MEASURED WITH *S. TYPHIMURIUM* STRAIN TA98 WITH HIGH (0.3 mL S9/mL S9 MIX), MEDIUM (0.1 mL S9 MIX) AND LOW (0.04 mL S9/mL S9 MIX)

		Level of Metabolic Activation		
Fraction CH_3OH	Dose/Plate (mg)	High 0.3 mL S9/mL Mix	Medium 0.1 mL S9/mL Mix	Low 0.04 mL S9/mL Mix
		---(Net his^+ Revertants/Plate (Mean ± S.D.)----		
AD2 Sludge	0.0	29 ± 13	31 ± 9	31 ± 6
	0.5	9 ± 3	7 ± 4	8 ± 4
	1.0	11 ± 4	6 ± 6	13 ± 6
	2.5	30 ± 9	14 ± 8	28 ± 4
	5.0	72 ± 12	60 ± 6	21 ± 8
	10.0	130 ± 31	99 ± 33	9 ± 15
Benzo(a) pyrene	.005	237 ± 70	196 ± 24	202 ± 42

The results presented in Table 5-7 are the dose response and concurrent solvent/positive control data for each of the 49 sludge fractions tested in TA98. These data provide a description of the mutagenic potential of the solvent extractable organics in each sludge fraction. The sludge fractions were tested at a relatively high dose

TABLE 5-7. MUTAGENICITY OF SOLVENT EXTRACTABLE ORGANIC FRACTIONS FROM VARIOUS SAMPLES OF SEWAGE SLUDGE

		Total TA98 his$^+$ Revertants (Mean ± SD)					
		CH_2Cl_2		CH_3OH		Combined	
Sample	Dose mg/plate	-S9	+S9	-S9	+S9	-S9	+S9
SM0	0.0	32 ± 4.8	32 ± 6.2	34 ± 3.7	40 ± 11.0	34 ± 3.7	40 ± 12.0
	0.1	16 ± 18.0	49 ± 16.0	14 ± 7.2	33 ± 2.4	25 ± 2.9	NT†
	0.5	29 ± 6.4	44 ± 12.0	30 ± 8.8	36 ± 7.4	25 ± 3.9	34 ± 3.4
	1.0	33 ± 3.2	60 ± 11.0	30 ± 2.1	41 ± 15.0	30 ± 9.1	30 ± 8.1
	2.5	35 ± 8.2	55 ± 16.0	35 ± 5.1	36 ± 3.9	31 ± 6.7	33 ± 6.2
	5.0	38 ± 7.2	74 ± 5.1	36 ± 5.1	42 ± 3.9	51 ± 7.8	37 ± 11.0
	10.0	47 ± 6.6	70 ± 13.0	35 ± 8.0	22 ± 8.3	21 ± 5.5	32 ± 9.4
SM1	0.0	32 ± 5.7	37 ± 5.8	32 ± 3.3	37 ± 4.2	32 ± 3.3	37 ± 4.2
	0.1	35 ± 7.8	41 ± 5.3	38 ± 7.1	43 ± 9.5	32 ± 5.8	40 ± 7.8
	0.5	41 ± 13.0	43 ± 5.1	32 ± 6.8	44 ± 8.9	37 ± 7.9	41 ± 4.8
	1.0	46 ± 21.0	44 ± 6.1	39 ± 5.6	36 ± 1.5	39 ± 8.2	52 ± 8.0
	2.5	44 ± 6.0	43 ± 5.5	33 ± 6.3	50 ± 10.0	38 ± 7.3	62 ± 16.0
	5.0	44 ± 2.6	92 ± 6.7	41 ± 7.9	73 ± 14.0	45 ± 11.0	98 ± 9.4
	10.0	52 ± 12.0	80 ± 22.0	23 ± 11.0	94 ± 10.0	20 ± 4.3	49 ± 2.0
SM2	0.0	32 ± 4.5	29 ± 9.4	32 ± 5.3	37 ± 3.7	32 ± 3.8	31 ± 12.0
	0.1	23 ± 3.6	31 ± 4.5	NT	NT	21 ± 8.8	24 ± 8.2
	0.5	24 ± 5.7	31 ± 8.1	30 ± 4.1	54 ± 20.0	20 ± 4.6	32 ± 5.7
	1.0	26 ± 11.0	38 ± 10.0	30 ± 12.0	58 ± 26.0	20 ± 6.8	37 ± 3.2
	2.5	NT	NT	31 ± 5.7	89 ± 54.0	NT	NT
	5.0	42 ± 24.0	47 ± 21.0	36 ± 6.0	115 ± 32.0	29 ± 10.0	33 ± 13.0
	10.0	43 ± 49.0	119 ± 11.0	49 ± 12.0	71 ± 45.0	41 ± 5.0	36 ± 10.0
SM3	0.0	32 ± 1.9	41 ± 2.7	33 ± 4.8	48 ± 7.0	27 ± 5.0	43 ± 12.0
	0.5	34 ± 12.0	39 ± 7.0	33 ± 3.0	52 ± 8.0	35 ± 13.0	57 ± 7.0
	1.0	44 ± 8.5	40 ± 11.0	34 ± 3.0	63 ± 9.0	45 ± 20.0	81 ± 17.0
	2.5	75 ± 5.7	52 ± 8.2	36 ± 5.0	97 ± 29.0	60 ± 16.0	78 ± 27.0
	5.0	84 ± 17.0	49 ± 8.4	43 ± 11.0	126 ± 48.0	110 ± 21.0	86 ± 16.0
	10.0	151 ± 14.0	66 ± 13.0	35 ± 3.0	135 ± 15.0	107 ± 13.0	114 ± 26.0

TABLE 5-7 CONTINUED.

Total TA98 his^+ Revertants (Mean ± SD)

Sample	Dose mg/plate	CH_2Cl_2		CH_3OH		Combined	
		-S9	+S9	-S9	+S9	-S9	+S9
SM4	0.0	35 ± 4.0	28 ± 4.0	36 ± 6.0	28 ± 4.0	36 ± 5.0	34 ± 3.0
	0.5	39 ± 14.0	32 ± 14.0	31 ± 6.0	20 ± 3.0	27 ± 9.0	28 ± 8.0
	1.0	55 ± 17.0	36 ± 19.0	25 ± 2.0	26 ± 11.0	34 ± 5.0	25 ± 7.0
	2.5	77 ± 21.0	30 ± 16.0	30 ± 10.0	31 ± 12.0	62 ± 11.0	45 ± 13.0
	5.0	128 ± 7.0	52 ± 15.0	41 ± 11.0	24 ± 8.0	92 ± 21.0	45 ± 9.0
	10.0	157 ± 16.0	49 ± 15.0	34 ± 13.0	31 ± 14.0	131 ± 16.0	62 ± 10.0
SM5	0.0	32 ± 5.4	41 ± 7.8	32 ± 5.4	41 ± 7.8	32 ± 5.4	41 ± 7.8
	0.5	25 ± 7.0	46 ± 17.0	22 ± 4.0	43 ± 10.0	22 ± 2.0	37 ± 4.0
	1.0	29 ± 4.0	48 ± 8.0	28 ± 7.0	39 ± 10.0	25 ± 4.0	39 ± 15.0
	2.5	23 ± 4.0	42 ± 8.0	26 ± 6.0	40 ± 18.0	28 ± 6.0	47 ± 13.0
	5.0	41 ± 5.0	61 ± 4.0	41 ± 6.0	52 ± 13.0	30 ± 1.3	66 ± 12.0
	10.0	45 ± 2.8	87 ± 8.0	40 ± 4.0	72 ± 10.4	37 ± 4.8	104 ± 8.0
SM6	0.0	32 ± 8.4	26 ± 5.2	32 ± 8.4	26 ± 5.2	32 ± 8.4	26 ± 5.2
	0.5	41 ± 9.7	30 ± 4.1	25 ± 3.3	24 ± 3.5	37 ± 9.2	25 ± 6.4
	1.0	73 ± 9.5	36 ± 8.4	24 ± 7.6	26 ± 7.8	49 ± 10.0	36 ± 8.7
	2.5	85 ± 8.3	49 ± 9.4	26 ± 5.8	34 ± 10.3	74 ± 9.2	48 ± 23.0
	5.0	65 ± 17.0	69 ± 6.9	34 ± 8.7	35 ± 8.3	97 ± 7.1	77 ± 28.0
	10.0	42 ± 10.0	99 ± 9.7	34 ± 15.0	37 ± 3.0	44 ± 9.2	98 ± 29.0
JT1	0.0	32 ± 3.3	37 ± 4.2	32 ± 3.3	37 ± 4.2	32 ± 3.2	37 ± 4.2
	0.1	34 ± 8.0	35 ± 9.7	14 ± 16.0	38 ± 7.6	31 ± 4.7	32 ± 3.8
	0.5	32 ± 8.1	43 ± 16.0	36 ± 8.4	30 ± 4.8	30 ± 3.8	36 ± 5.6
	1.0	25 ± 16.0	47 ± 2.4	37 ± 8.2	28 ± 4.9	33 ± 6.1	45 ± 5.1
	2.5	33 ± 8.1	53 ± 8.9	36 ± 7.8	36 ± 12.0	30 ± 7.8	44 ± 5.1
	5.0	47 ± 10.0	53 ± 35.0	30 ± 4.8	41 ± 8.5	48 ± 11.0	26 ± 18.0
	10.0	15 ± 2.5	32 ± 4.6	20 ± 4.0	29 ± 7.0	NT	56 ± 10.0

TABLE 5-7 CONTINUED

Sample	Dose mg/plate	Total TA98 his$^+$ Revertants (Mean ± SD) CH_2Cl_2 -S9	CH_2Cl_2 +S9	CH_3OH -S9	CH_3OH +S9	Combined -S9	Combined +S9
VG1	0.0	29 ± 3.1	35 ± 7.1	29 ± 3.1	35 ± 7.1	NT	NT
	0.1	28 ± 7.5	24 ± 7.0	25 ± 9.6	33 ± 11.0	NT	NT
	0.5	29 ± 7.8	38 ± 19.0	30 ± 6.2	42 ± 12.0	NT	NT
	1.0	30 ± 17.0	44 ± 14.0	36 ± 14.0	63 ± 14.0	NT	NT
	2.5	25 ± 3.9	50 ± 14.0	35 ± 22.0	82 ± 9.5	NT	NT
	5.0	35 ± 10.0	93 ± 14.0	52 ± 15.0	119 ± 17.0	NT	NT
	10.0	NT	155 ± 11.0	NT	NT	NT	NT
BN	0.0	29 ± 3.1	35 ± 7.1	29 ± 3.1	35 ± 7.1	29 ± 3.1	35 ± 7.1
	0.5	30 ± 8.5	45 ± 10.0	34 ± 1.7	43 ± 13.0	33 ± 6.2	45 ± 11.0
	1.0	40 ± 3.3	51 ± 15.0	30 ± 9.1	42 ± 10.0	33 ± 14.0	51 ± 13.0
	2.5	38 ± 6.1	61 ± 13.0	34 ± 9.9	41 ± 8.6	30 ± 15.0	47 ± 12.0
	5.0	35 ± 6.3	52 ± 15.0	38 ± 14.0	56 ± 21.0	36 ± 12.0	59 ± 19.0
	10.0	45 ± 7.9	83 ± 12.0	41 ± 7.4	58 ± 16.0	37 ± 8.5	74 ± 7.2
RD	0.0	31 ± 4.3	31 ± 4.6	31 ± 4.3	31 ± 4.6	32 ± 5.3	37 ± 3.7
	0.5	23 ± 8.8	41 ± 18.0	23 ± 6.6	25 ± 6.9	32 ± 5.9	39 ± 17.0
	1.0	26 ± 2.5	42 ± 4.3	25 ± 5.2	32 ± 7.3	27 ± 7.7	57 ± 14.0
	2.5	28 ± 8.2	39 ± 26.0	25 ± 9.2	29 ± 8.1	23 ± 5.3	71 ± 5.9
	5.0	NT	51 ± 13.0	21 ± 5.3	38 ± 13.0	NT	47 ± 36.0
	10.0	NT	NT	27 ± 8.8	50 ± 19.0	NT	Tox**
ST	0.0	36 ± 1.6	32 ± 7.7	36 ± 1.6	32 ± 4.7	25 ± 5.7	29 ± 3.0
	0.5	31 ± 4.3	35 ± 12.0	39 ± 18.0	39 ± 6.7	24 ± 4.5	32 ± 10.0
	1.0	35 ± 5.0	35 ± 9.0	32 ± 14.0	42 ± 12.0	23 ± 7.4	29 ± 5.2
	2.5	34 ± 5.4	33 ± 6.0	27 ± 13.0	28 ± 2.2	29 ± 14.0	40 ± 11.0
	5.0	34 ± 12.0	35 ± 14.0	32 ± 13.0	27 ± 7.3	44 ± 3.9	44 ± 17.0
	10.0	31 ± 10.0	33 ± 10.0	36 ± 11.0	31 ± 9.3	Tox	38 ± 15.0

TABLE 5-7 CONTINUED

Sample	Dose mg/plate	Total TA98 his$^+$ Revertants (Mean ± SD)					
		CH_2Cl_2		CH_3OH		Combined	
		-S9	+S9	-S9	+S9	-S9	+S9
TA	0.0	32 ± 4.9	26 ± 6.6	32 ± 4.9	32 ± 4.7		
	0.5	25 ± 5.4	35 ± 20.0	28 ± 7.4	NT	NT	NT
	1.0	36 ± 12.0	32 ± 3.4	38 ± 6.0	45 ± 14.0	NT	NT
	2.5	24 ± 4.3	62 ± 3.4	41 ± 7.1	62 ± 16.6	NT	NT
	5.0	27 ± 2.9	86 ± 11.0	19 ± 3.7	56 ± 6.6	NT	NT
	10.0	48 ± 4.1	69 ± 37.0	43 ± 4.1	99 ± 18.0	NT	NT
SK	0.0	32 ± 4.9	31 ± 4.5	32 ± 4.9	31 ± 4.5	25 ± 5.7	31 ± 5.8
	0.5	20 ± 4.6	44 ± 28.0	26 ± 8.6	31 ± 5.7	16 ± 0.8	51 ± 32.0
	1.0	19 ± 5.0	47 ± 22.0	21 ± 8.0	53 ± 9.0	20 ± 5.5	65 ± 3.9
	2.5	25 ± 5.2	66 ± 14.0	22 ± 5.7	77 ± 5.9	18 ± 5.1	36 ± 15.0
	5.0	16 ± 3.7	69 ± 23.0	18 ± 4.5	68 ± 0.96	29 ± 10.0	39 ± 14.0
	10.0	NT	37 ± 18.0	29 ± 8.9	65 ± 18.0	28 ± 12.0	31 ± 2.0
AD1	0.0	32 ± 3.6	37 ± 7.5	32 ± 3.6	37 ± 7.5	32 ± 3.6	37 ± 7.5
	0.5	28 ± 7.5	41 ± 2.3	22 ± 1.8	40 ± 8.0	28 ± 2.0	40 ± 11.0
	1.0	26 ± 5.3	39 ± 5.3	25 ± 5.3	58 ± 13.0	24 ± 4.0	58 ± 12.0
	2.5	30 ± 5.3	55 ± 6.3	22 ± 5.3	79 ± 22.0	27 ± 3.0	75 ± 1.0
	5.0	37 ± 4.5	74 ± 5.0	27 ± 3.3	126 ± 10.0	29 ± 5.0	124 ± 8.0
	10.0	54 ± 10.0	95 ± 7.0	32 ± 6.0	224 ± 11.0	42 ± 8.0	172 ± 42.0
AD2	0.0	25 ± 5.0	35 ± 4.0	25 ± 5.0	35 ± 4.0	25 ± 5.0	35 ± 4.0
	0.5	21 ± 4.0	45 ± 6.0	17 ± 4.0	39 ± 3.0	19 ± 5.0	31 ± 4.0
	1.0	25 ± 7.0	52 ± 5.0	21 ± 3.0	41 ± 6.0	26 ± 6.0	30 ± 2.0
	2.5	22 ± 6.0	59 ± 5.0	22 ± 6.0	55 ± 11.0	22 ± 5.0	54 ± 4.0
	5.0	25 ± 3.0	80 ± 3.0	28 ± 11.0	105 ± 16.0	22 ± 4.0	84 ± 5.0
	10.0	29 ± 8.0	83 ± 9.0	29 ± 4.0	146 ± 7.0	21 ± 6.0	125 ± 6.0

TABLE 5-7 CONTINUED

Sample	Dose mg/plate	Total TA98 his$^+$ Revertants (Mean ± SD)					
		CH_2Cl_2		CH_3OH		Combined	
		-S9	+S9	-S9	+S9	-S9	+S9
JT2	0.0	34 ± 3.5	36 ± 4.8	31 ± 4.3	31 ± 6.6	32 ± 5.3	37 ± 3.7
	0.5	24 ± 8.4	26 ± 9.6	27 ± 8.4	27 ± 7.5	34 ± 10.0	49 ± 2.9
	1.0	25 ± 3.9	39 ± 15.0	30 ± 13.0	41 ± 13.0	32 ± 7.4	65 ± 12.0
	2.5	21 ± 1.6	45 ± 13.0	27 ± 9.6	52 ± 25.0	23 ± 6.2	56 ± 8.8
	5.0	37 ± 8.2	53 ± 8.0	38 ± 7.1	71 ± 18.0	30 ± 7.9	77 ± 13.0
	10.0	47 ± 14.0	77 ± 18.0	33 ± 15.0	77 ± 22.0	37 ± 7.5	55 ± 8.5
Historical Control Values							
None	-	30 ± 8	40 ± 15				
DMSO	0.05	26 ± 6	37 ± 10				
2NF	0.025	2197 ± 445	NT				
BAP	0.005	28 ± 7	609 ± 152				

†NT = Not tested; tox = toxic; None = no additions to top agar; DMSO = dimethyl sulfoxide; 2NF = 2-nitrofluorene; BAP = benzo(a)pyrene.

levels (up to 10 mg per plate) because much of the solvent extractable organic material was believed to be inert or non-reactive in the microbial mutagenicity assay. The maximum dose suggested in the EPA's standard procedures for the Salmonella bioassay is also 10 mg/plate (EPA,1985). The sludge fractions displayed a tremendous amount of variability in their capacity to induce TA98 mutations. While only indirect-acting mutagens were detected in the majority of the sludge fractions, direct acting mutagens were detected in two samples (SM3 and SM4); and, none of the fractions from sludge samples JT-1, BN, RD, and ST induced a positive response (Table 5-7). It is possible that the absence of mutagenic activity in the ST sludge is a result of the efforts of the city to limit the quantity of hazardous constituents in the sewage system (Galvin, 1987).

Chemicals extracted in the CH_2Cl_2 fraction should represent the more non-polar sludge components. Sludge samples for which the CH_2Cl_2 fraction failed to induce a doubling of revertants at two consecutive dose levels include the SM2, JT1, BN, ST, and JT2 sludges. At a dose level of 5 mg per plate with activation, the CH_2Cl_2 fraction of SM0 and SM1 induced 74 and 92 total revertant colonies or slightly greater than two times background (Table 5-7). The fourth sample from the SM wastewater treatment plant, SM3, induced 151 total revertant colonies (4.7 times background) at a dose level of 10 mg per plate without activation. In the presence of metabolic activation, the mutagenic response induced by the CH_2Cl_2 fraction of the SM3 sludge was reduced or completely eliminated. Although the VG sludge had one of the lowest concentrations of solvent extractable organics of the sludge samples tested, the mutagenic potential of the CH_2Cl_2 fraction of this sludge was 155 total revertants (4.4 times backgound). Since this sample had been stored in drying beds for several months, it is possible that the magnitude of this response is a result of the presence of partially degraded sludge mutagens. The maximum mutagenic response induced by the CH_2Cl_2 fraction with metabolic activation was 157 total revertants (4.5 times background) induced by the SM4 sludge at a dose of 10 mg per plate. Thus, the mutagenic potential of the CH_2Cl_2 fractions extracted from twelve different sludges ranged from a low of 3 net revertants to a high of 122 net revertants per 10 mg of residue.

The solvent extractable organics contained in the CH_3OH fractions should include the majority of the polar or water soluble constituents. The CH_3OH fraction from seven of the twelve sludges, including SM0, SM1, JT1, BN, ST, and TA failed to induce a doubling of revertant colonies at two consecutive dose levels with or without metabolic activation (Table 5-7). At a dose level of 10mg per plate with metaboblic activation, the CH_3OH fraction of the SK sludge induced 65 total revertants (2.1 times background) while the JT2 sludge induced 77 total revertants (2.5 times background). With metabolic activation, the CH_3OH fraction of the VG sludge induced 119 total revertants (3.4 times background) at a dose of 5 mg per plate (Table 5-7). The maximum mutagenic response observed in a CH_3OH fraction was 227 total revertants (6.1 times background) induced by the AD1 sludge at a dose of 10 mg per plate with activation (Table 5-7). For the CH_3OH fractions, the response induced in the bioassays ranged from 2 net revertants to 187 net revertants. In addition, for all twelve sludges tested, a mutagenic response was observed with the CH_3OH fraction only in

The weighted activity of each of the fractions from the various sludges is presented in Table 5-8, Figures 5-6 and 5-7. This value was obtained by multiplying the specific activity (net revertants mg^{-1}) from Table 5-7, times the residue weight (mg g^{-1}) from Table 5-1 for each fraction and allows for a comparison of sludge samples based on equivalent weights. The results indicate that six of the seventeen sludges (SM0, JT1, VG, RD, ST, and TA) contained relatively low quantities (less than 100 revertants per gram) of organic mutagens. Although the specific activity of the fractions of the VG sludge were quite high, the weighted activity is relatively low due to the low quantity of solvent extractable organics in the sludge. Moderate levels (between 100 and 350 revertants per gram) of organic mutagens were detected in the sludges from JT2, SM1, SM2, SM5, BN, and SK. Relatively high weighted activities (greater than 350 revertants per gram) were observed in sludge samples SM3, SM4, SM6, AD1, and AD2. The weighted activities with activation for the AD sludges were 168, 430, and 702 revertants per gram for the CH_2Cl_2, CH_3OH and combined fractions of the AD1 sludge, respectively; while, the CH_2Cl_2, CH_3OH and combined fractions of the AD2 sludge induced 211, 278, and 621 revertants per gram, respectively. Without metabolic activation, the CH_2Cl_2 fraction of the SM3 sludge induced 631 revertants per gram, and the combined fraction induced 736 revertants per gram, while the CH_3OH fraction induced 340 revertants per gram with activation, and less than 8 without activation. The maximum weighted activity observed was 1197 revertants per gram in the combined fraction of the SM4 sludge without activation, while the CH_2Cl_2 fraction induced 767 revertants per gram without activation, and the

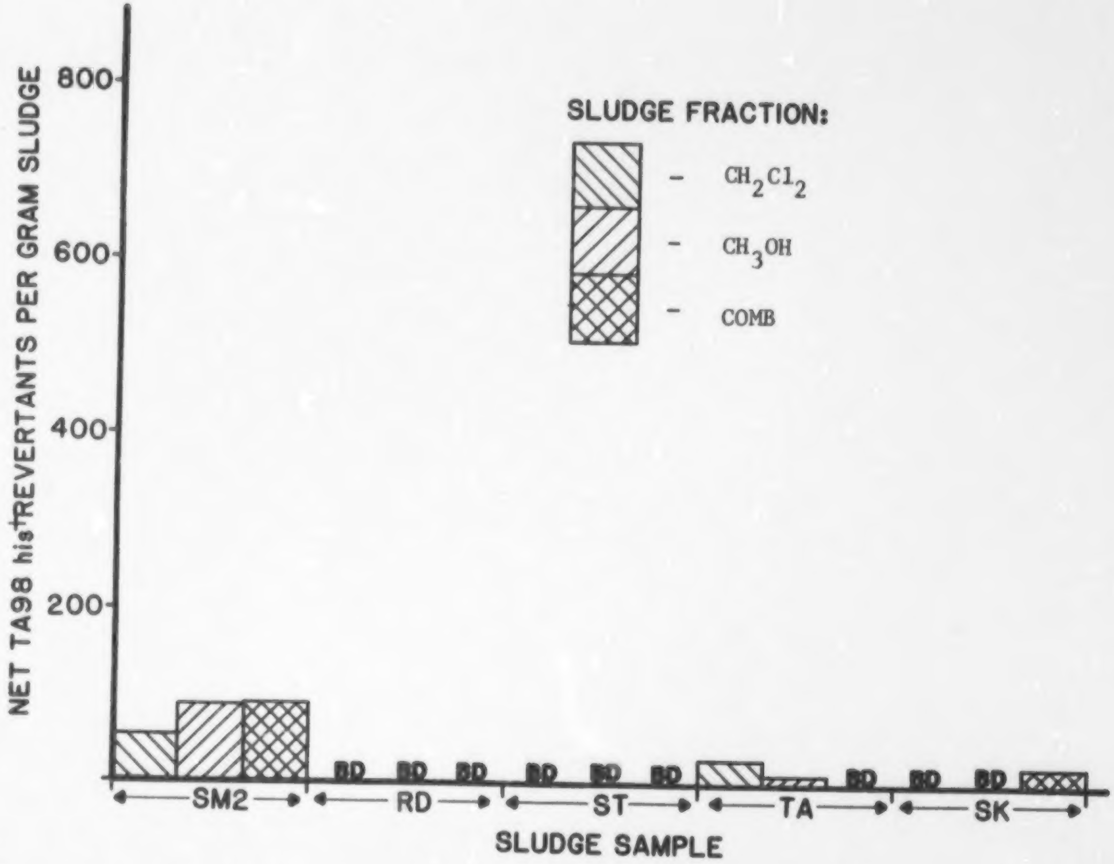


Figure 5-6. Weighted activity without metabolic activation of methylene chloride (CH_2Cl_2), methanol (CH_3OH), and combined fractions (COMB) extracted from various samples of municipal sewage sludge.

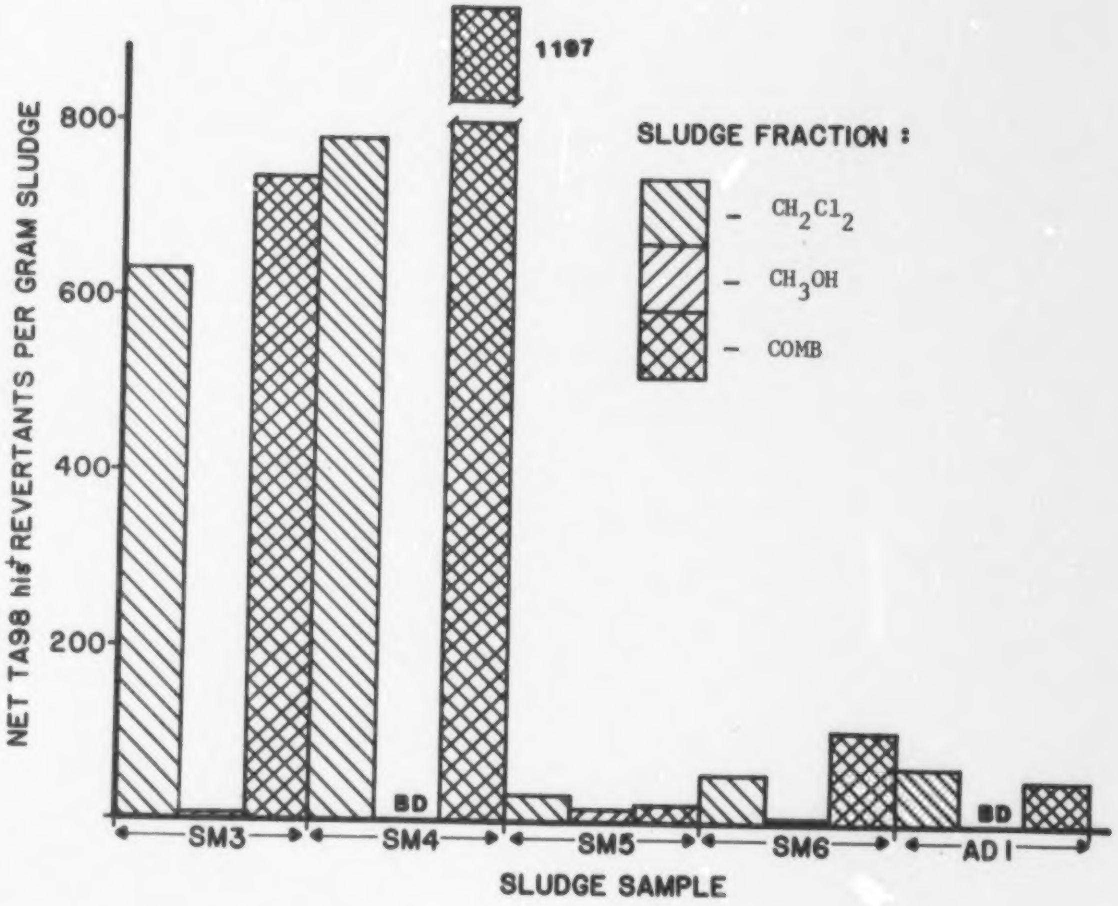


Figure 5-6 continued.

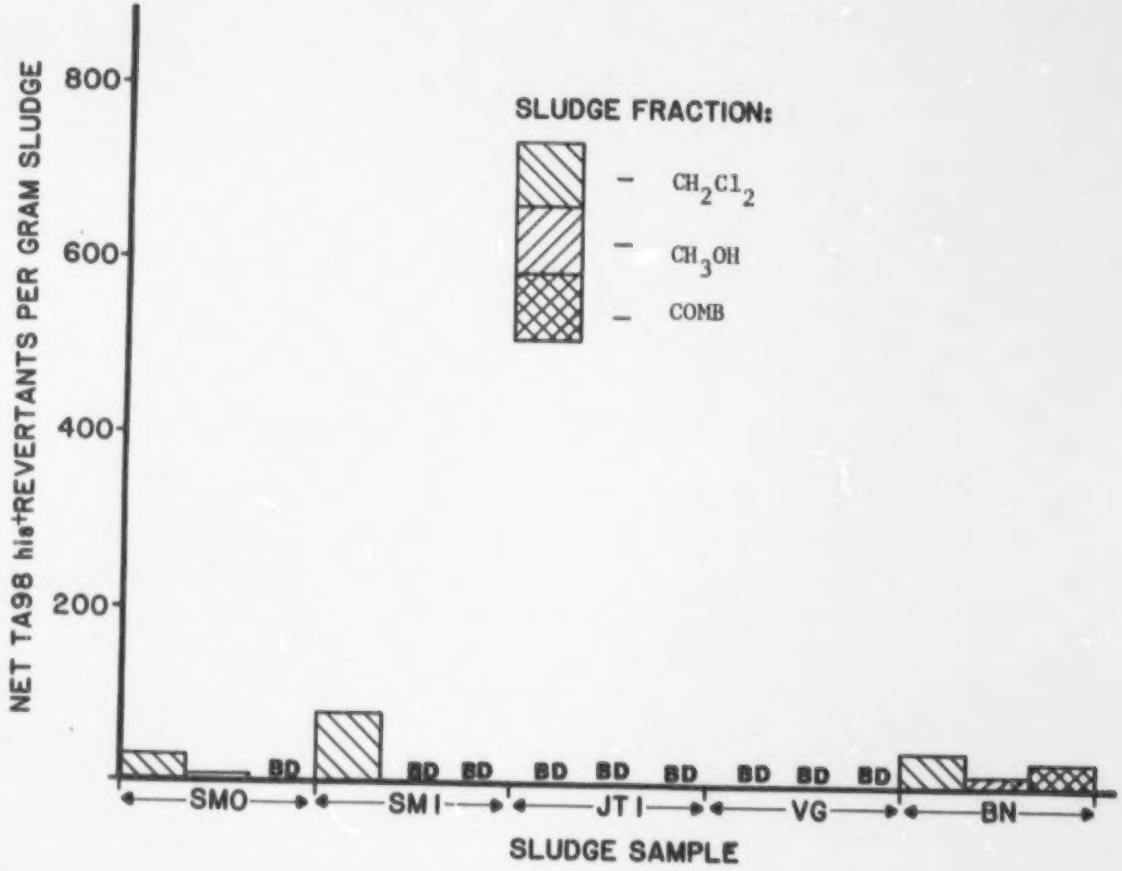
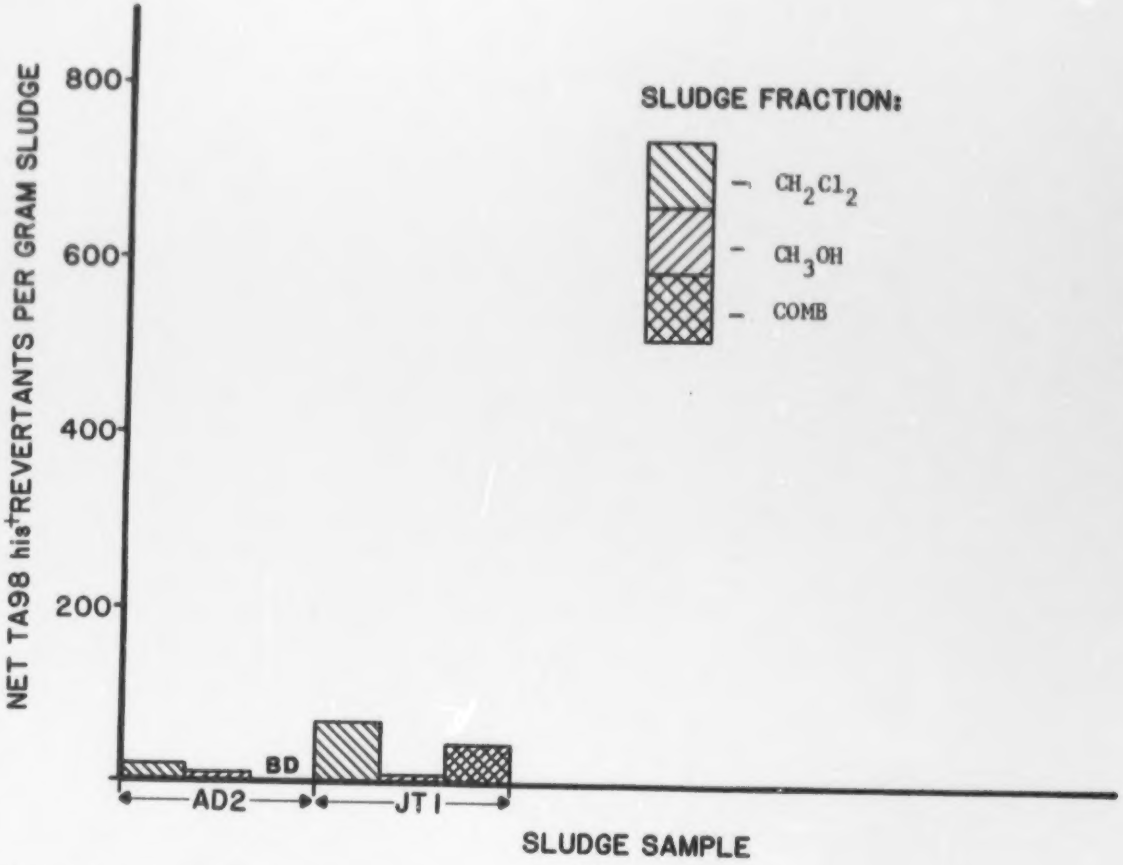


Figure 5-6 continued.



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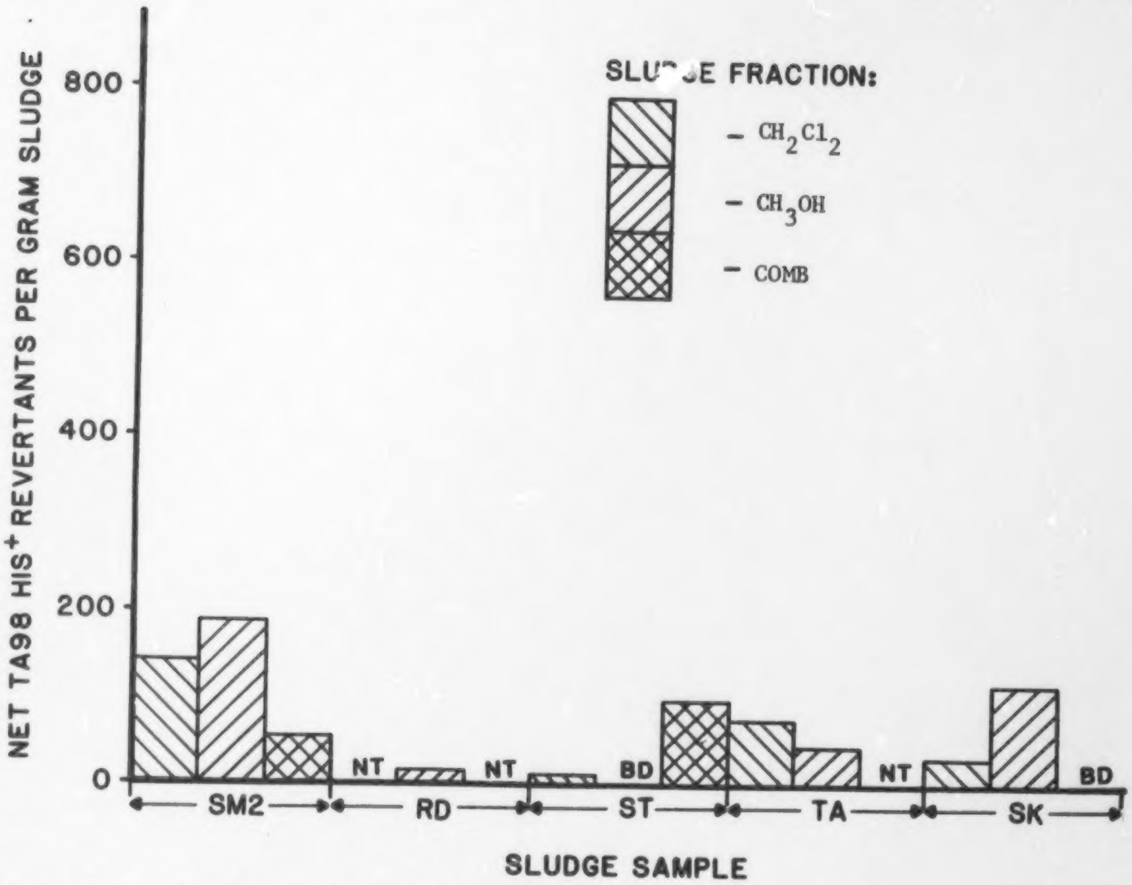
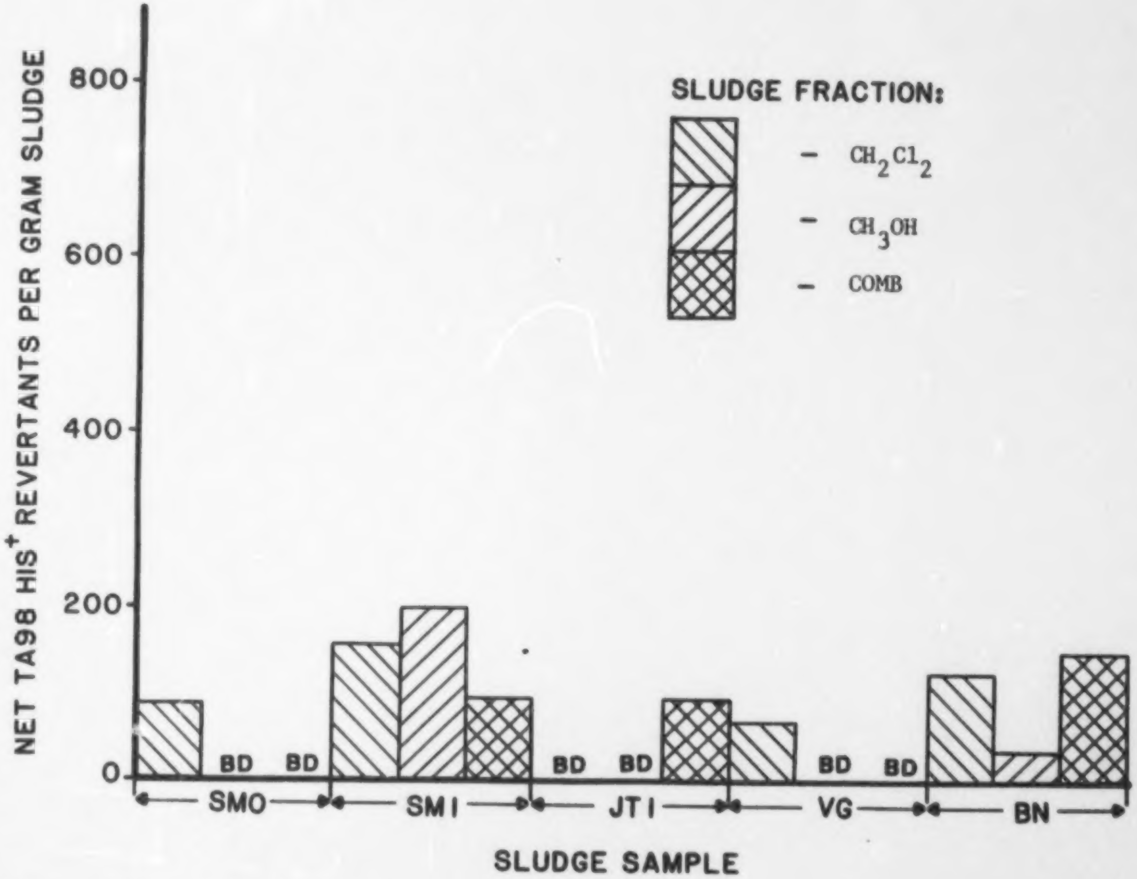


Figure 5-7. Weighted activity with metabolic activation of methylene chloride (CH_2Cl_2), methanol (CH_3OH), and combined fractions (COMB) from various samples of municipal sewage sludge.



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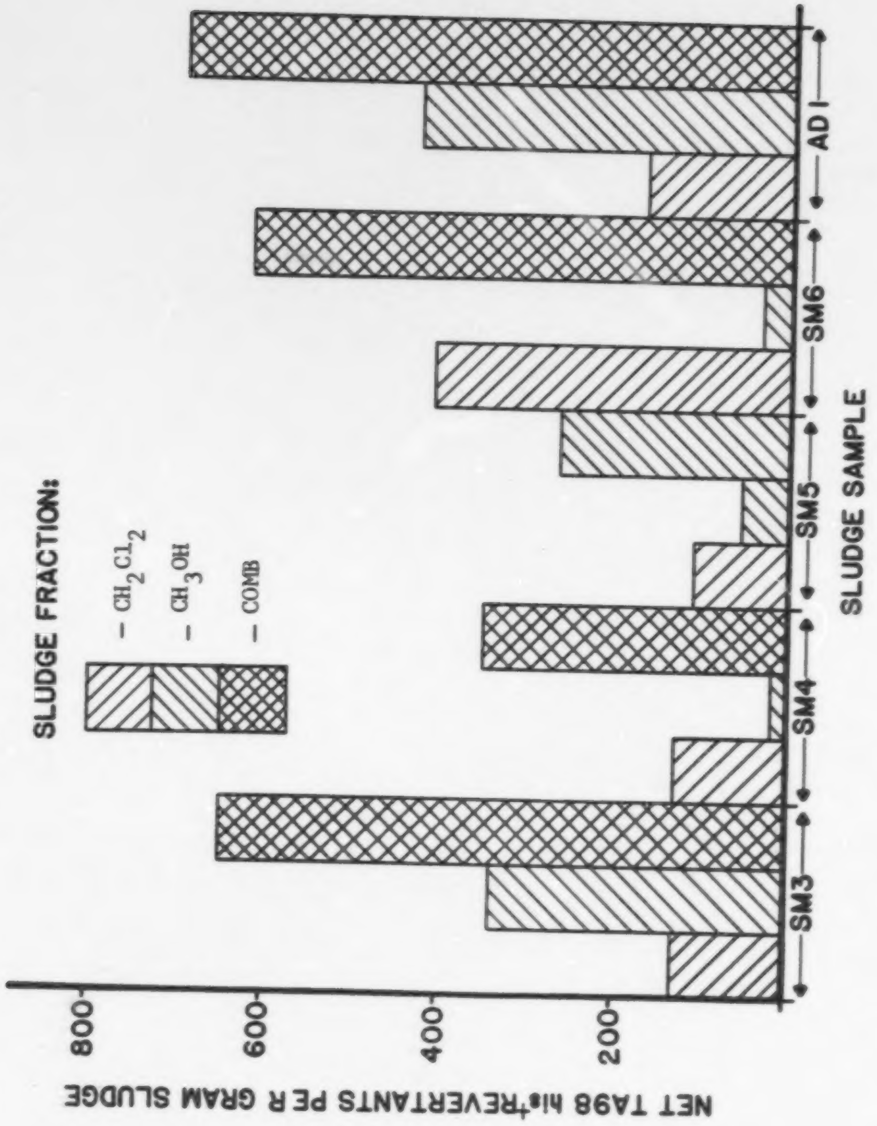

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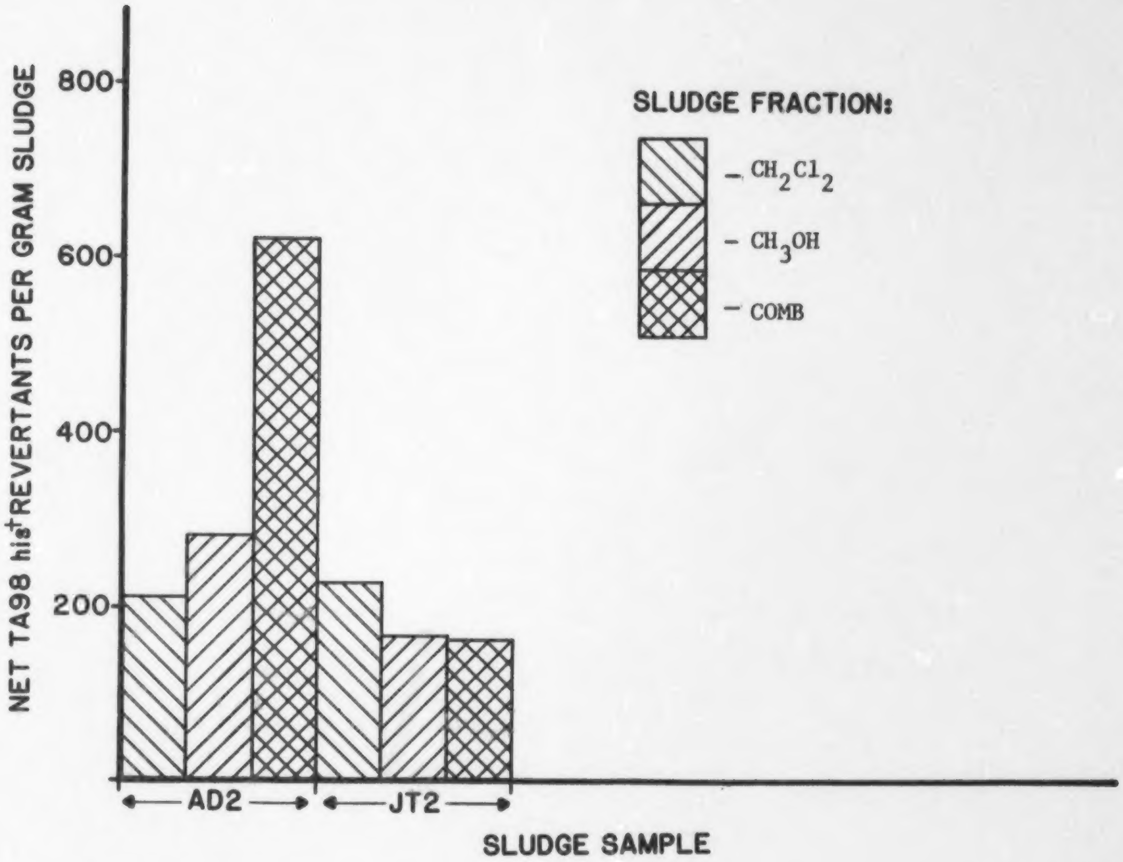

Figure 5-7 continued.

TABLE 5-8. SUMMARY OF SPECIFIC AND WEIGHTED ACTIVITIES FOR VARIOUS FRACTIONS OF MUNICIPAL SEWAGE SLUDGE

Sample	Specific Activity†						Weighted Acitivity††					
	CH_2Cl_2		CH_3OH		Combined		CH_2Cl_2		CH_3OH		Combined	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
SM0	15	38	1	0	0	0	32	81	.003	BD	BD	BD
SM1	20	43	0	57	0	12	82	176	BD	198	BD	91
SM2	11	90	17	34	9	5	54	145	90	180	92	51
SM3	119	25	2	87	80	71	631	133	8	339	736	653
SM4	122	21	0	3	95	28	769	132	BD	19	1197	353
SM5	13	46	8	31	5	63	31	110	14	56	21	265
SM6	10	73	2	11	12	72	56	409	6	33	103	619
JT1	0	0	0	0	NT	19	BD	BD	BD	BD	BD	86
VG	NT	120	NT	NT	NT	NT	BD	61	BD	BD	BD	BD
BN	16	48	12	23	8	39	41	124	15	28	30	148
RD	NT	NT	0	19	NT	TOX	NT	NT	BD	13	NT	NT
ST	0	1	0	0	TOX	29	NT	7	BD	BD	BD	93
TA	16	43	11	67	NT	NT	28	75	7	45	NT	NT
SK	NT	6	0	34	3	0	BD	33	BD	109	26	BD
AD1	22	58	0	187	10	135	64	168	BD	430	52	702
AD2	4	48	4	111	0	90	18	211	10	278	BD	621
JT2	13	41	2	46	5	18	72	226	7	166	46	164

† Specific activity equals net TA98 his^+ revertants per 10 mg residue (from Table 5-7).

†† Weighted activity equals specific activity divided by 10 (net revertants/mg) times residue weight (mg residue per gram sludge from Table 5-1).

BD Below detection; NT = not tested; TOX = toxic.

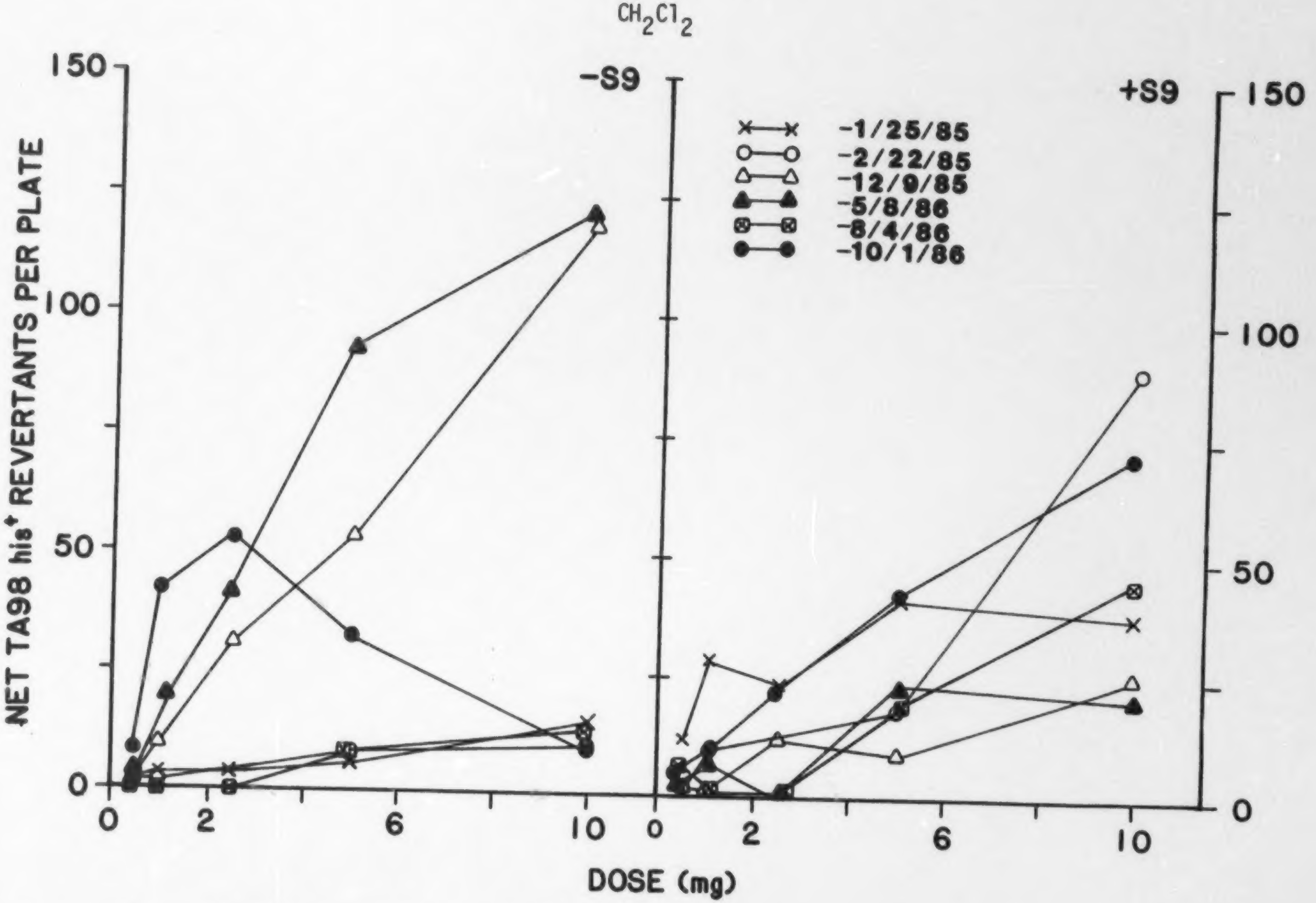

Figure 5-8. Mutagenic potential of methylene chloride (CH_2Cl_2) fraction extracted from six samples of SM sludge collected over a 20 month period.

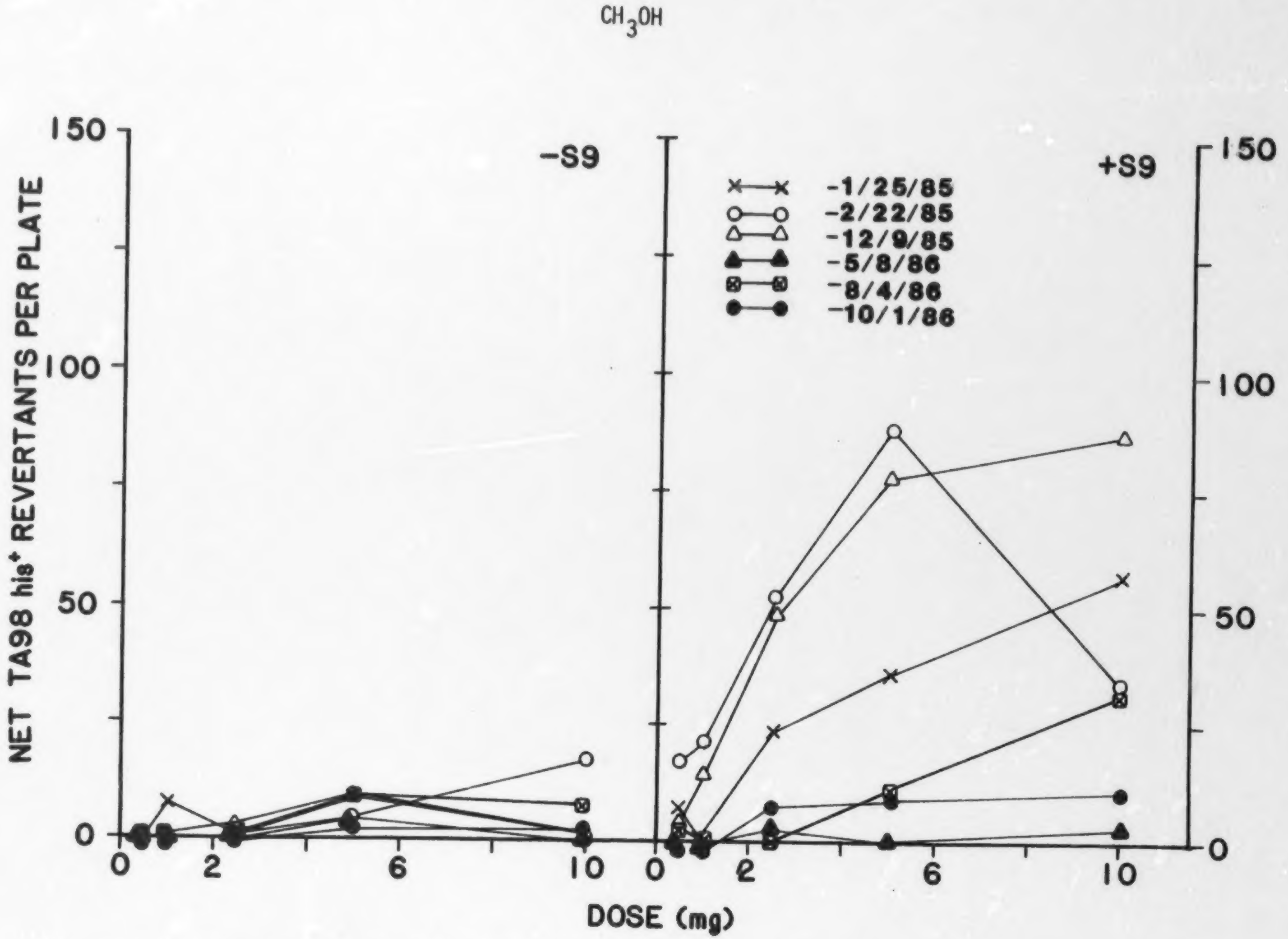

Figure 5-9. Mutagenic potential of methanol (CH_3OH) fraction extracted from six samples of SM sludge collected over a 20 month period.

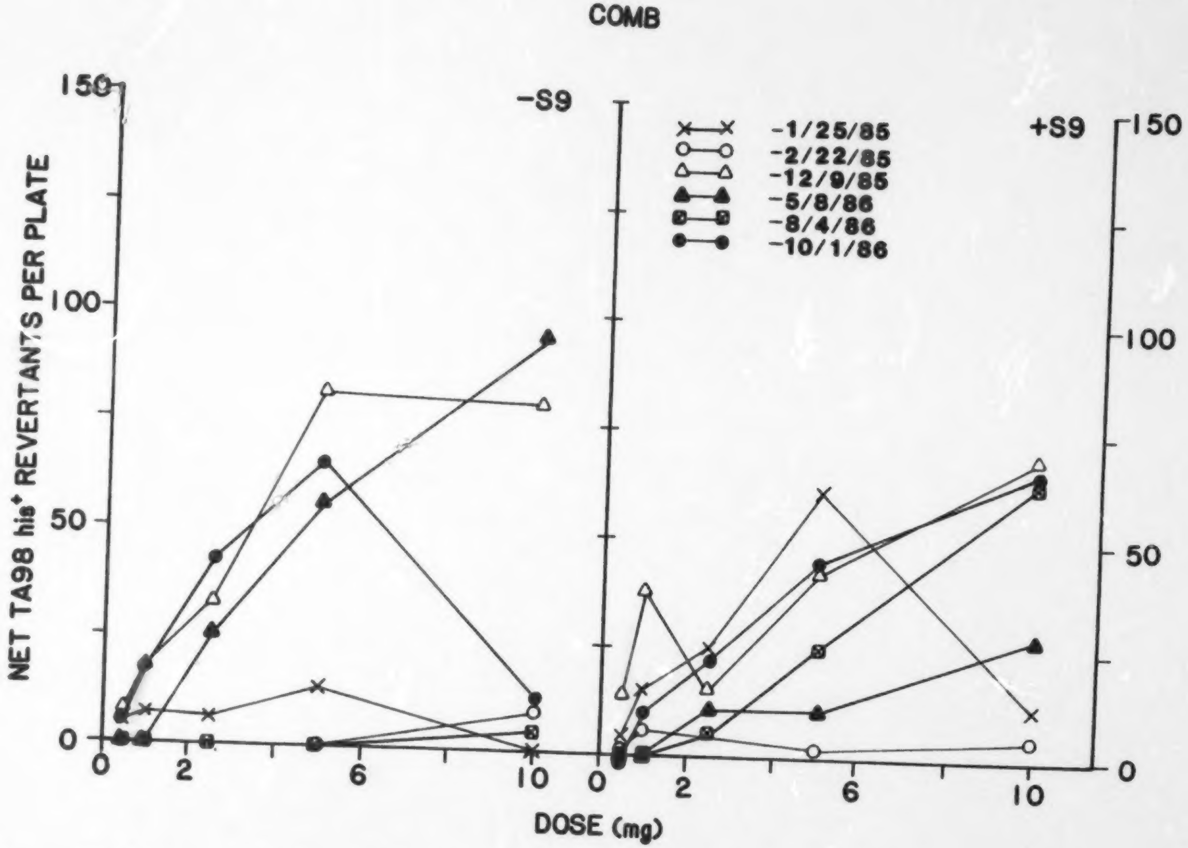

Figure 5-10. Mutagenic potential of combined (COMB) fraction extracted from six samples of SM sludge collected over a 20 month period.

revertants at two consecutive dose levels (Figure 5-8). In the presence of metabolic activation, only the CH_2Cl_2 fraction of sample SM6 collected 10/1/86 induced a positive response. While, in the absence of metabolic activation, none of the CH_3OH fractions of the sludge samples from the SM plant induced a mutagenic response. With metabolic activation, the samples SM2 (2/22/86) and SM3 (12/9/86) induced a weak mutagenic response. The combined fraction from three sludge samples (SM3-12/9/85; SM4-5/8/86; and SM6-10/1/86) induced a positive response without metabolic activation (Figure 5-10).

Thus, in the eighteen fractions from six separate sludge samples collected over a twenty month period both direct and indirect acting mutagens were detected. Only one sample failed to induce a positive response in any of the three fractions with or without metabolic activation. Predominately direct acting mutagens were detected in the CH_2Cl_2 fraction, while indirect acting mutagens were detected in the majority of the CH_3OH fractions, and both classes of mutagens were detected in the combined fraction, although the magnitude of the response was usually greatest in the absence of metabolic activation. These data indicate that considerable variability may be anticipated in both the quantity and quality of organic mutagens in the sludge from any individual municipal wastewater treatment plant.

The weighted activities for the SM sludges ranged from below detection for fractions of SM0 sludge, to 1197 revertants per gram without activation for the combined fraction of the SM4 sludge (Table 5-8). All sludge samples contained at least one fraction which induced a weighted activity of 80 revertants per gram or greater. For the first three sludge fractions collected, the weighted activity was predominantly detected with metabolic activation in the CH_2Cl_2 and CH_3OH fractions. Samples SM3 and SM4 contained predominantly direct acting mutagens with the maximum responses observed in the CH_2Cl_2 and combined fractions. The last two samples collected, SM5 and SM6, were again found to contain primarily indirect acting mutagens in the CH_2Cl_2 fraction, although the weighted activity of the combined fraction was one-half to two times greater than that of the CH_2Cl_2 fraction. Thus, although appreciable levels of organic mutagens were detected in all sludge samples, the maximum weighted activity was more than twelve times greater than the weighted activity of the least active sludge sample.

CONCLUSIONS

The data presented above provides an indication of the mutagenic potential of three fractions from seventeen different samples of sewage sludge. These data and information reported in the literature indicate that a tremendous variability can be anticipated between samples of sludge from different sources, as well as different samples of sludge from the same source. In the present study, the specific activity of sludge fractions ranged from below detection to 187 net revertants per 10 mg of residue (approximately five times background); while, the weighted activity ranged from below detection to 1197 revertants per gram of sludge (Table 5-8). For comparative purposes, a Weswood soil and a wood preserving waste induced a specific activity of 67 and 141 net revertants per milligram, respectively; and, a weighted activity of 2 revertants per gram for the

Weswood soil, and 20,070 revertants per gram for the wood preserving waste (Brown *et. al.*, 1985 and Donnelly *et. al.*, 1987b). Several studies reported in the literature indicate that most sludge samples contain relatively small quantities of organic mutagens. Clevenger *et al.* (1983) reported no mutagenic reponse in five samples investigated, while Angle and Baudler (1984) also reported no response greater than twice the concurrent solvent control. Hopke *et al.* (1984) evaluated the mutagenic potential of five sewage sludges and observed a positive response from only one of the five samples tested. The crude extract from the Sauget sludge induced 3,363 revertants at a dose of 4.2 mg per plate (Hopke *et al.*, 1984), while Johnston *et al.* (1983) also observed a strong positive response for the sludge from the Sauget treatment plant. Johnston *et al.* (1983) found that all ten of the sludges evaluated in their study induced a positive response in the Salmonella/microsome assay. A study by Tabor *et al.* (1983) evaluated different extraction procedures for municipal wastewaters and observed a maximum response of 251,000 net revertants per gram dry weight. However, since dose response data are not provided it is difficult to compare these results with those in the present study. A more recent study by Johnson and Baker (1986) detected a maximum response of four times background in the sludge samples tested. The results of the present study, as well as those reported in the literature indicate that there may be a significant difference in the level of organic mutagens in different samples of municipal sewage. The data also indicate that multiple samples from a single source may display a large variance in the level of mutagenicity. Thus, it appears that bioassays can serve as an indicator of the mutagenic activity of municipal sludges, although chemical analysis is an important tool to identify the components of active samples.

SECTION 6

ENVIRONMENTAL FATE OF LAND APPLIED SEWAGE SLUDGE

Approximately seven million dry tons of sewage sludge are generated in the United States each year (EPA, 1983). Common disposal methods include ocean dumping, landfilling, incineration, and land application. As environmental and energy restrictions limit the use of other alternatives, many municipalities are evaluating the utility of land application. A recent survey indicated that approximately 40% of municipal sewage is ultimately disposed of by some form of land application (EPA, 1983). A major environmental concern associated with land application of municipal sewage sludge is the ultimate fate of sludge borne organics. A partial list of organic chemicals which have been identified in samples of sewage sludge (presented in Table 1-1) includes a diverse range of organic chemicals including several which are established mutagens (McCann *et al*., 1975; Scott *et al*., 1982).

Angle and Baudler (1984) monitored the persistence and degradation of mutagens in sludge amended soil and observed that the mutagenicity of the sludge amended soil increased during the initial seven days of the study, and rapidly decreased after seven days. However, the sludge and sludge amended soil did not induce a response that was greater than twice the concurrent solvent control, and no dose response data was provided for the one mutagenic sample that was obtained from this study. Thus, it is difficult to determine the true importance of this data. In a separate study, Johnston et al. (1986) applied sewage sludge to soil and observed that more than seven weeks were required for the mutagenic activity of the sludge amended soil to reach a maximum, and that the activity of the soil returned to background levels rapidly thereafter.

The present study was conducted to monitor the fate of organic mutagens in soil. This section of the present research project employed undisturbed monolith lysimeters to monitor the fate and mobility of municipal sewage sludge applied to two different soils at three application rates.

MATERIALS AND METHODS

Sludge

Samples were collected from a single wastewater treatment plant for the land application study on three separate occasions (December 9, 1985; May 8, 1986; August 4, 1986) and included samples SM3, SM4, and SM5. All of these samples were dried sludges obtained from a C. E. Raymond cage mill flash drier. The treatment process used to generate these sludges is described in more detail for the SM plant in Section 4 and a general summary of the mutagenic potential of the various sludge fractions is provided in Table 6-1.

Soils

Two soils were employed to represent a range in soil textures. The general characteristics of the soils are listed in Table 6-2, and a summary of their mutagenic properties is provided in Table 6-1 (Brown and Donnelly, 1984; Donnelly and Brown, 1981). Particle size distribution was measured by the pipette technique of Gee and Bauder (1986). Organic matter was measured by wet combustion (Nelson and Sommers, 1982). CEC was

TABLE 6-1. GENERAL CHARACTERISTICS OF SOILS AND SEWAGE SLUDGE SAMPLES USED IN FIELD STUDY

Sample	Fraction	Solvent Extractable Organics (mg/g)	Specific Activity (rev/mg)		Weighted Activity (rev/mg)	
			-S9	+9	-S9	+9
Padina Sand	Methylene Chloride	0.57	13	10	7	6
	Methanol	0.46	27	5	12	2
	Combined	1.03	10	3	10	3
Weswood Loam	Methylene Chloride	0.16	11	11	2	2
	Methanol	1.31	32	11	42	14
	Combined	1.48	13	9	19	13
SM3 (Dec)	Methylene Chloride	5.3	119	25	631	133
	Methanol	3.9	2	87	8	340
	Combined	9.2	80	71	736	653
SM4 (May)	Methylene Chloride	6.3	122	21	769	132
	Methanol	6.3	0	3	BD	19
	Combined	12.6	95	28	1197	353
SM5 (Aug)	Methylene Chloride	2.4	13	46	31	110
	Methanol	1.8	8	31	14	56
	Combined	4.2	5	63	21	265

TABLE 6-2. PHYSICAL PROPERTIES OF THE TWO SOILS

Soil	Sand	Silt	Clay	Texture	pH	Solvent Extractable Organics
	------ g kg^{-1} --------					
Weswood	482	152	366	SC	7.7	1.08
Padina	818	45	144	SL	6.5	0.71

determined by the ammonium saturation method (Rhoades, 1982). Soil reaction (pH) was measured in a 1:1 (soil:water) suspension using a glass electrode (McLean, 1982). Solvent extractable organic carbon was determined using CH_2Cl_2 and CH_3OH following procedures of Brown et al. (1984).

The Weswood silty clay (Fluventic Ustochrept) was obtained from the Texas A&M University Research Farm. This soil had been fallow for four to five years prior to being cropped to a sorghum-sudan hybrid. The Weswood soil has a Class I land use capability which indicates that this series has very few limitations and can be used for intensive cultivation practices. Potential sources of mutagenic contaminants in the Weswood soil include exhaust from the tractors and direct application or drift of biocides from sprayed crops. A previous study of the bacterial mutagenicity of the Weswood soil revealed that the solvent extractable organics from this soil induced 2 revertants per gram of soil extracted (Brown et al., 1986b).

The Padina loamy sand (Grossarenic Paleustalfs) was collected from a pasture area adjacent to the Brazos River. The area from which this soil was collected had been used as a pasture for grazing cattle for many years prior to sample collection. Thus, the potential for chemical contamination is anticipated to be relatively low.

Lysimeters

Twenty-four undisturbed soil monolith lysimeters (twelve each of the Weswood and Padina soils) were used in this portion of the study. A detailed description of the procedure for the collection and installation of lysimeters is given by Brown et al. (1974). Briefly, the technique is as follows: Casings (28cm x 90cm) were obtained from the Sharney Container Corp. (Houston, Texas). These 55 gallon straight-walled barrels were cleaned and coated with an epoxy based heavy metal free paint (Shertar, Sherwin Williams Co.). Using an iron frame for support, the casings were forced into the ground with pressure applied by a backhoe. As the casing was pushed into the ground, the soil was removed around the outside to relieve the pressure. The backhoe was used to lift the soil monolith and to roll it upside down. Sufficient soil was removed from the bottom of the casing to install three porous ceramic suction cups at the bottom of the soil profile. The nylon tubes that were used to conduct the leachate to the surface were threaded through a 1.27 cm diameter PVC tube installed along the inside of one wall. A gasket was sealed in place, and the bottom was clamped on the casing. The lysimeters were then turned upright and installed in a trench approximately 80 cm deep. The soil was then filled around the outside of the lysimeters to help maintain a typical thermal regime. A channel of soil approximately 10 cm wide and 15 cm deep was removed from the inner edge of each lysimeter and duct tape placed in an "L" shape around the inside of the lysimeter to prevent side wall flow. Following the installation of the duct tape, the soil was replaced in the trench.

To establish the integrity of each undisturbed soil monolith lysimeter, approximately 20L of a solution containing a 100 mg L^{-1} Br^- as KBr spike was applied to each lysimeter. The breakthrough of the Br to the ceramic suction cups installed at the bottom of the soil profile was then used as a measurement of the integrity of the lysimeter. Breakthrough is determined by measuring the effluent Br concentration (C) and dividing this value by the influent Br concentration (C_o). If C/C_o is greater than 0.5 before 0.5 pore volumes have been collected, this indicates that the soils are not behaving as a homogenous porous media and flow is occuring through structural cracks or other channels. However, if breakthrough occurs after the collection of 0.3 pore volumes for the Weswood soil, or 0.5 pore volumes for the Padina soil, it is assumed that the soil monolith is not cracked and does not have a channel which will allow side wall flow. The data presented in Figures 6-1 through 6-8 represent the Br breakthrough curves for all 24 lysimeters employed in this research. Lysimeters 3, 4, and 11 (Padina sand with 150 Mg/ha) displayed breakthrough of the Br after 0.5 pore volumes of leachate were collected (Figure 6-1). For lysimeters 2, 6, and 8 (Padina sand with 50 Mg/ha) breakthrough was observed at 0.5 pore volumes or greater, and breakthrough did not occur until after 0.5 pore volumes of leachate had been collected from lysimeters 1, 9, and 10 (Padina sand with 100 Mg/ha) (Figures 6-2 and 6-3). For the Padina sand control lysimeters (Lysimeters 5, 7, and 12), breakthrough again occurred after the first 0.5 pore volumes of leachate had been collected (Figure 6-4).

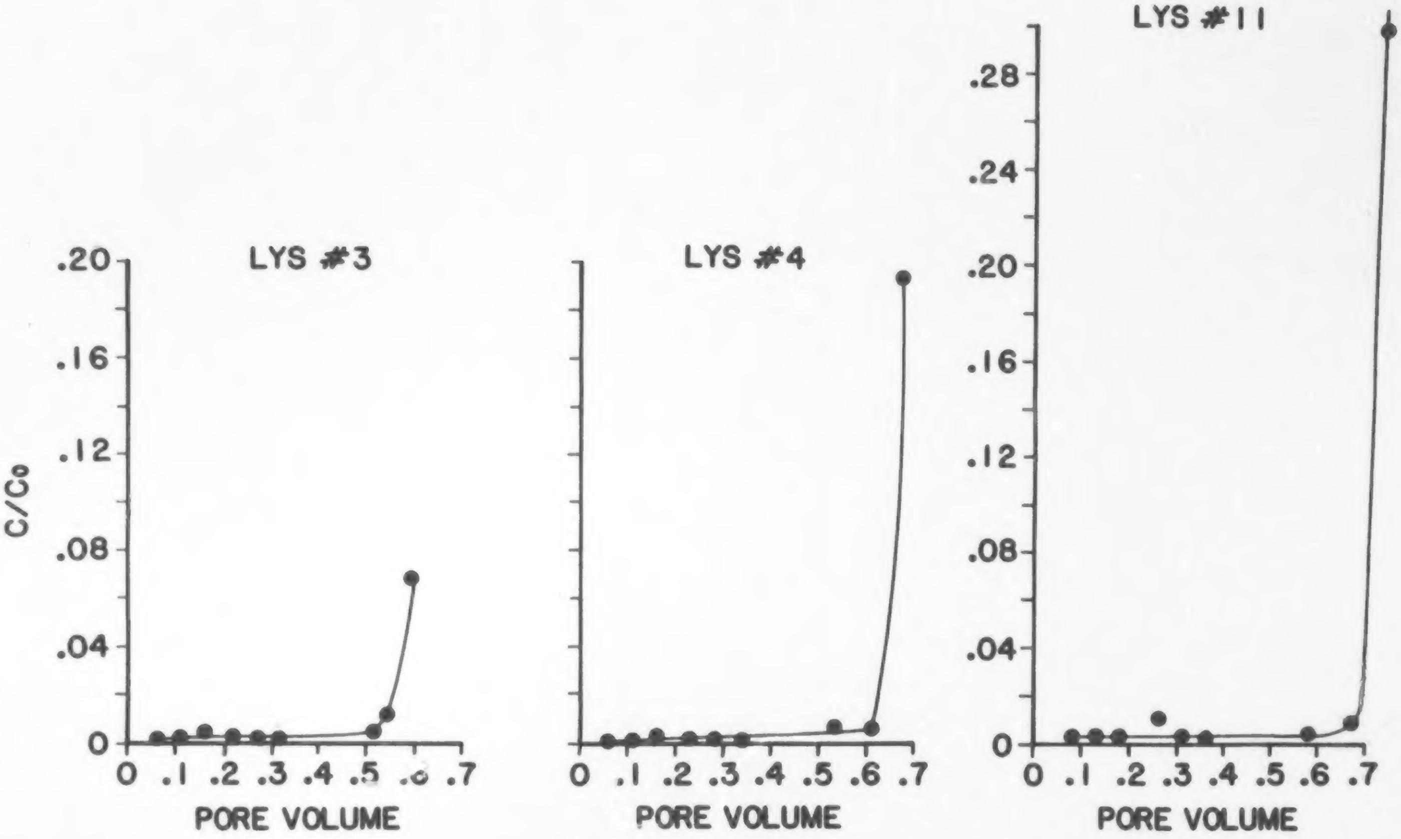

Figure 6-1. Bromide breakthrough curves for Padina sand lysimeters Nos. 3, 4 and 11.

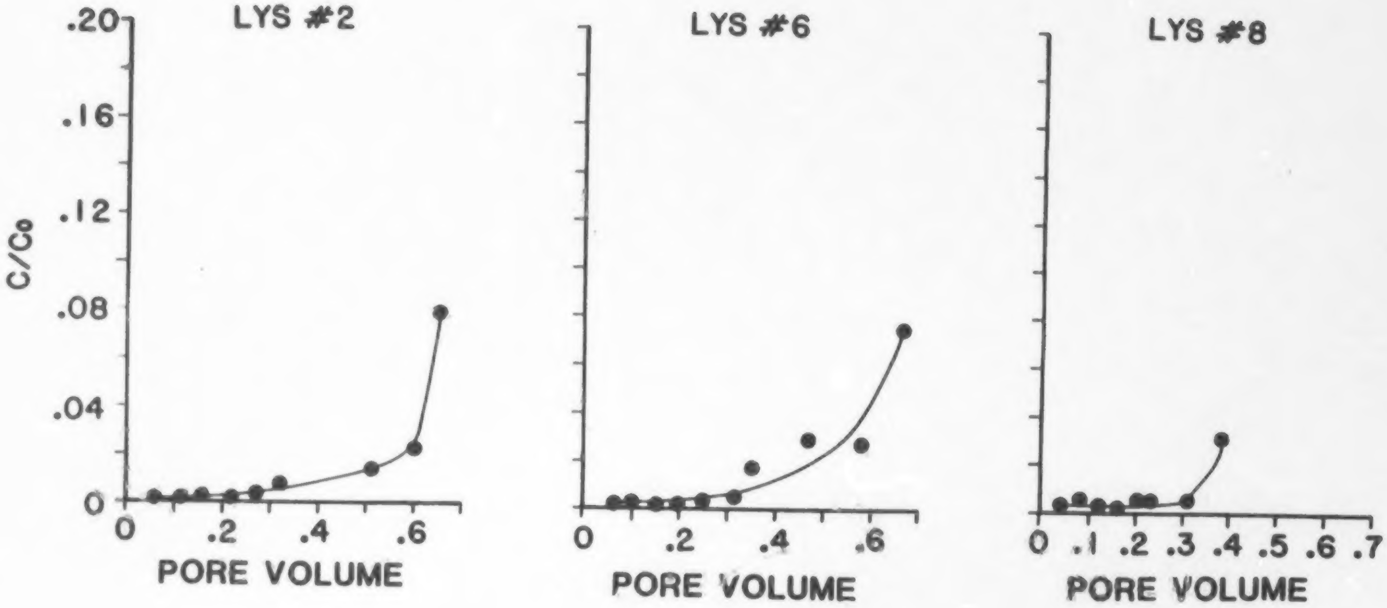

Figure 6-2. Bromide breakthrough curves for Padina sand lysimeters Nos. 2, 6, and 8.

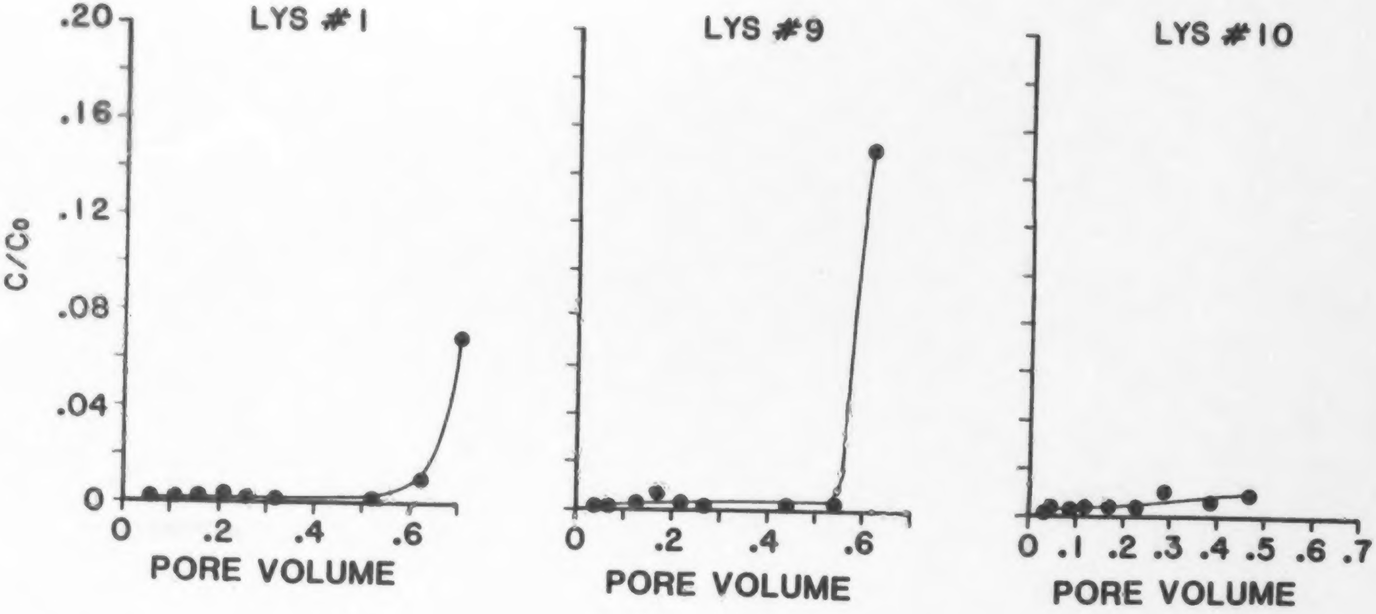

Figure 6-3. Bromide breakthrough curves for Padina sand lysimeters Nos. 1, 9, and 10.

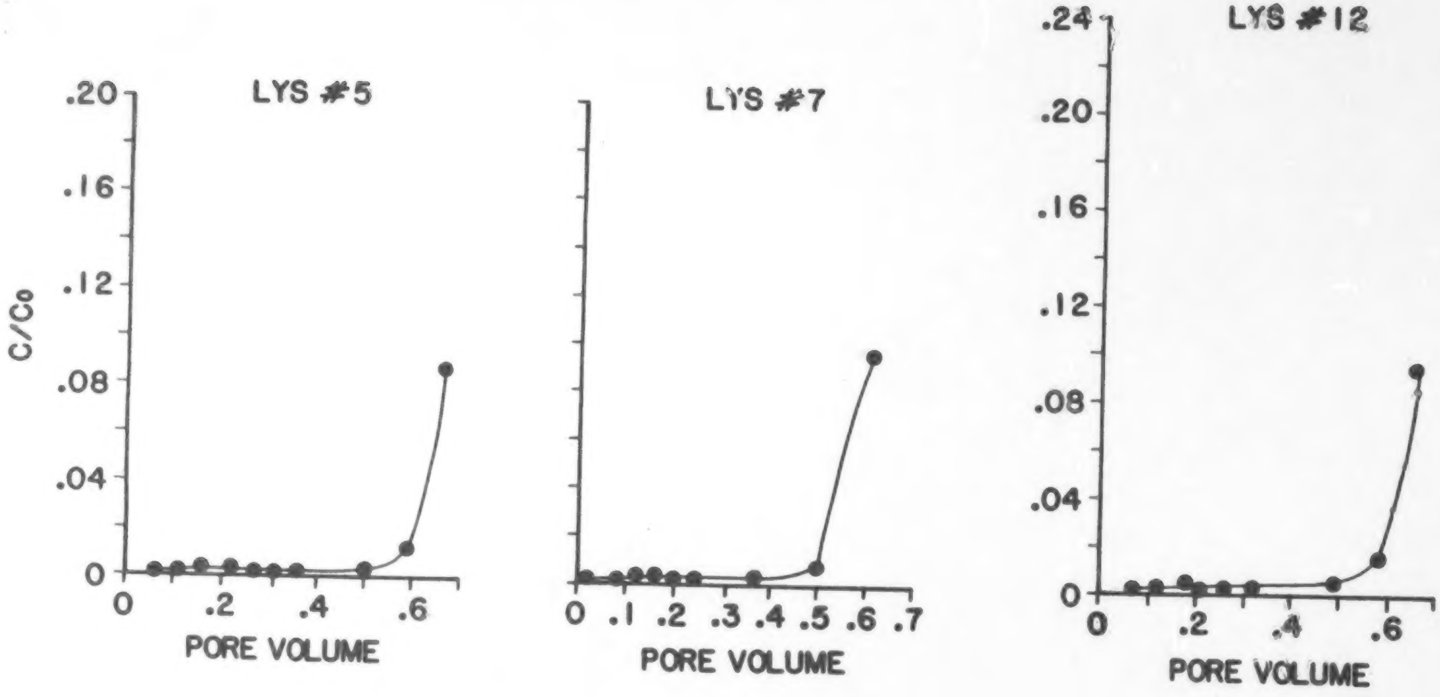

Figure 6-4. Bromide breakthrough curves for Padina sand lysimeters Nos. 5, 7, and 12.

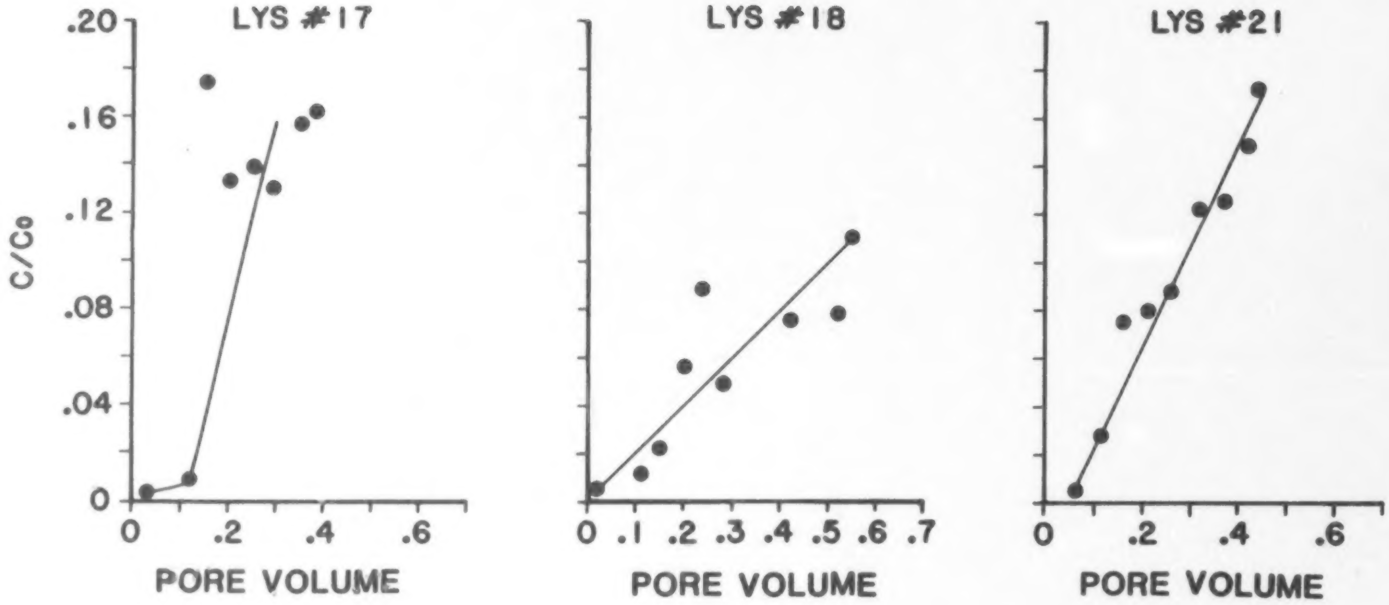

Figure 6-5. Bromide breakthrough curves for Weswood loam lysimeters Nos. 17, 18 and 21.

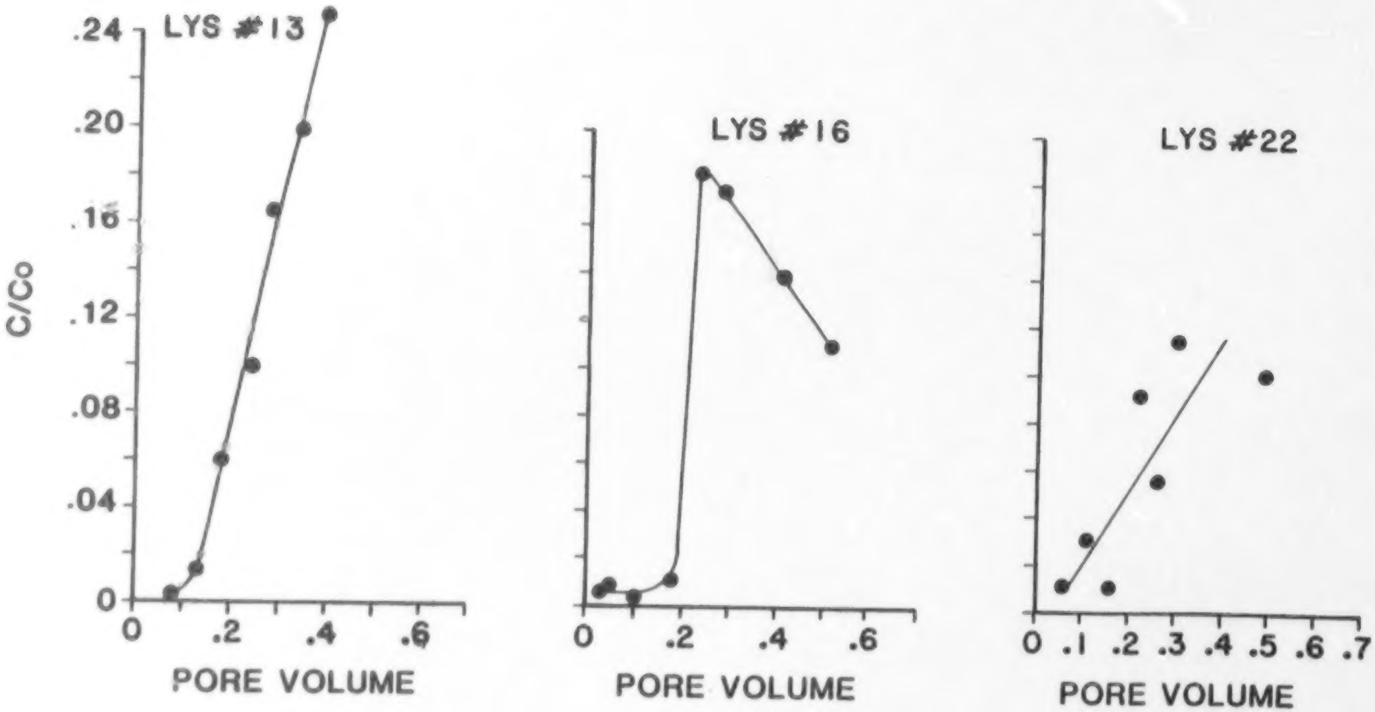

Figure 6-6. Bromide breakthrough curves for Weswood loam Lysimeters 13, 16, and 22.

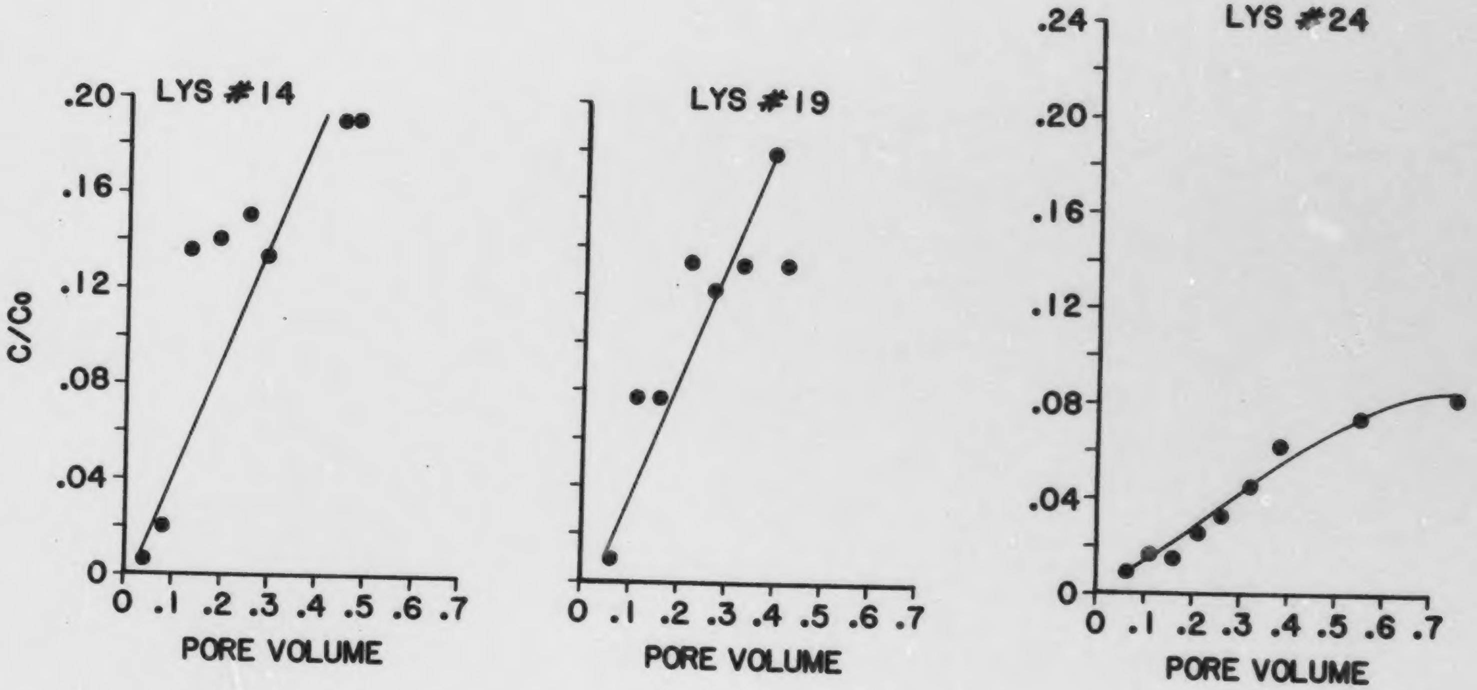

Figure 6-7. Bromide breakthrough curves for Weswood loam lysimeter Nos. 14, 19 and 24.

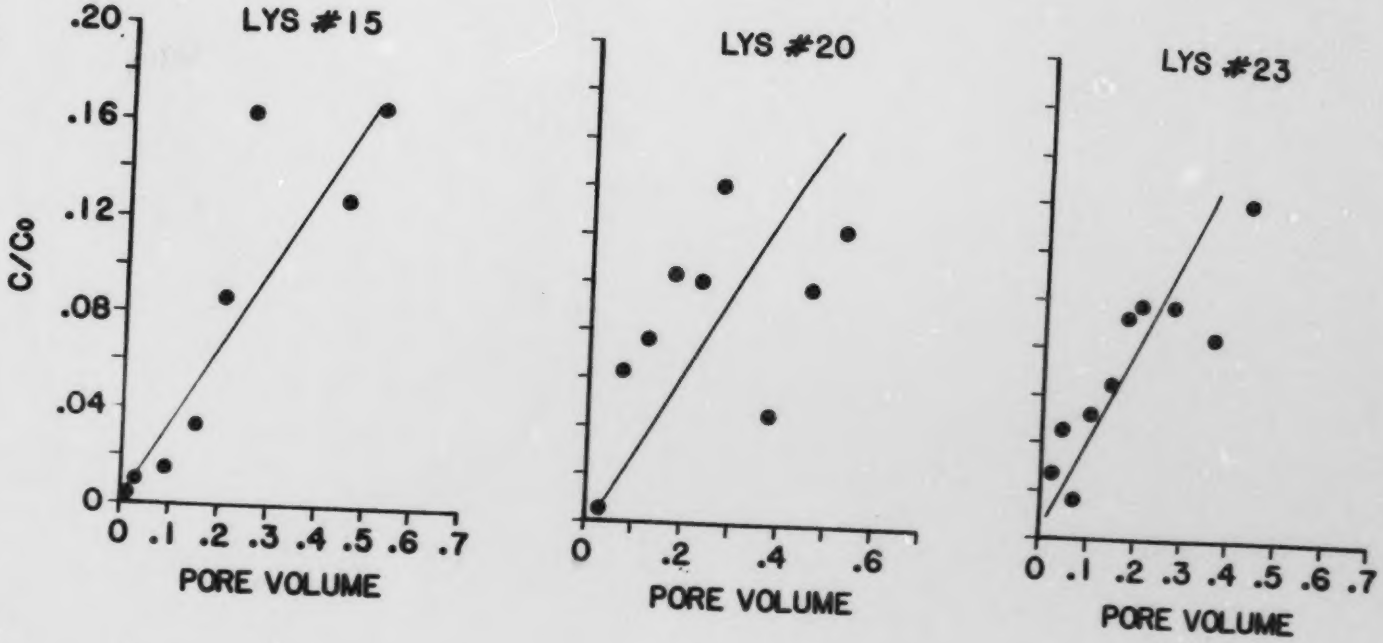

Figure 6-8. Bromide breakthrough curves for Weswood loam lysimeter Nos. 15, 20 and 23.

For the Weswood loam lysimeters which received two applications totalling 150 Mg/ha (Lysimeters 17, 18, and 21) C/C_o was not greater than 0.5 until after the first 0.4 pore volumes were collected (Figure 6-5). For the Weswood lysimeters which received one application of 50 Mg/ha (Lysimeters 13, 16, and 22), breakthrough occurred after 0.3 pore volumes of leachate were collected (Figure 6-6); while for the Weswood lysimeters which received one application of 100 Mg/ha (Lysimeters 14, 19, and 24), breakthrough was observed in the sample collected after 0.4 pore volumes had been collected (Figure 6-7). For the Weswood control lysimeters (Lysimeters 15, 20 and 23), breakthrough did not occur until after the collection of the first 0.5 pore volume of leachate (Figure 6-8). Thus, breakthrough for the Padina sand lysimeters occurred after approximately 0.5 pore volumes of leachate were collected, while a minimum of 0.4 pore volumes of leachate were collected from the Weswood loam lysimeters prior to breakthrough. This difference in flow rates was anticipated because the Weswood soil is more structured than the Padina.

Application Rates

The application rate of sewage sludge on agricultural land is limited by the content of heavy metals in the sludge, the nitrogen mineralization of the organic N, and nitrification of both the mineralized N and the NH_4^+ already present in the sludge at the time of application. The loading rates for heavy metals were established by Smith et al. (1983). Mineralization of organic nitrogen in sludge has been reported at 20% for the first year by Hinsley et al. (1974) and Harrison and Goodson (1974) and at 35% for the first year by Pratt et al. (1973). Losses of NH_4^+-N through

NH_3-volatilization can be as high as 50% of the NH_4^+-N in the sludge (Beauchamp et al., 1978). Nitrification of 20 to 50% of the N in sewage sludge can occur (Peterson et al., 1973). Leaching of large amounts of NO_3^- in the ground water can be the limiting factor in determining loading rates for certain sludges. Sludge loading rates should not result in quantities of NO_3-N in the ground water being greater than 10 mg L^{-1}. Specific loading rates must be determined by analyses of the sludge to be applied. However, general recommendations of rates of application exist. Hinsely et al. (1974) proposed that sludge loadings should be limited to less than 15 metric tons/ha, while Sommers (1977) proposed rates as high as 50 Mg/ha on a dry basis. Application rates adopted for this study included 50 and 100 Mg/ha or 5.5 and 11.0 kg/lysimeter, respectively.

The amount of sludge applied to each lysimeter was based on the volume of soil in the surface to 10 cm soil layer (6158 cm^3) and was determined by multiplying the weight of soil times the desired sludge application rate for each lysimeter. Six lysimeters (3, 4, 11, 17, 18, 21) received 11 kg (100 Mg/ha) on December 9, 1985; while on May 8, 1986, six lysimeters received 11 kg or 100 Mg/ha, (1, 9, 10, 14, 19, 24), and six received 5.5 kg or 50 Mg/ha (2, 6, 8, 13, 16, 22). The final sludge application took place on August 4, 1986, when six lysimeters received a second application of 5.5 kg or 50 Mg/ha (3, 4, 11, 17, 18, 21) for a total sludge application of 150 Mg/ha.

Soil Samples

Soil samples representing the 0 to 15 cm depth were collected from each lysimeter prior to and immediately after sludge application.

each lysimeter prior to and immediately after sludge application. Additional samples were collected 3, 7, 14, 28, 56, and 175 days after sludge application. For the May and August sludge applications, the three and seven day samples were excluded. A minimum of 250 g of soil were collected from each lysimeter on each sampling date. A precleaned metal trowel was used to collect six to ten randomly selected plugs (approximately 50 g each) from each lysimeter. Sufficient sample was collected to conduct a biological and chemical analysis on the soil extract, as well as to provide a reserve sample for future use. Precautions were taken to prevent cross-contamination of different depths of the core samples. These included cleaning the sampling device between corings, collecting samples from control before sludge amended lysimeters, and collecting multiple composited samples from each lysimeter.

Soil Extraction Procedure

The soil samples collected from the lysimeters were extracted using the Tecator Soxtec apparatus. Fifteen to 20 g samples were weighed and added to the cellulose extraction thimbles following the same procedures as used for the sludge samples. The thimbles were placed in the thimble support and attached to the thimble holder. The glass cups were filled with 25 mL of CH_2Cl_2 or CH_3OH and clamped into the condensors. Each sample was extracted for 5 min in the boiling position and 55 min in the rinsing position. After the rinsing time was completed, the condensor valves were closed and the residual solvent evaporated. All extractions were conducted in triplicate and composited. Two fractions were thus sequentially collected from each dried sample, a CH_2Cl_2 and a CH_3OH fraction. The residue from each extraction was then weighed, redissolved in dimethyl sulfoxide (DMSO-Grade I, Sigma Chem. Co., St. Louis, MO), and an aliquot of each fraction combined in equal portions to represent a third composite fraction.

Leachate Sample Collection

A schematic diagram of the leachate collection system is shown in Figure 6-9. Leachate from the bottom of each lysimeter was collected through three porous ceramic suction cups (Coors Type #70001-P-6-C). Each cup was tested in the laboratory prior to installation and had a bubbling pressure of 0.5 bars, a conductivity of 1.2 mL cm^{-2} min^{-1} bar^{-1}, and a surface area of 38.8 cm^{-2}.

Plexiglass caps were sealed to the open end of the cups with a water proof two part epoxy (Armstrong No. 34). Nylon tubes (0.159 cm diameter) were cemented into the plexiglass caps with another two part epoxy (Armstrong No. 6). The three tubes passed through the 1.27 cm PVC pipe to a plexiglass collection manifold approximately 7.5 cm above the soil surface. Nylon tubes of 0.32 cm diameter were used to convey the leachate to amber glass bottles in a refrigerator maintained at 4°C. A continuous vacuum was maintained on all twenty-four bottles. Each bottle was equipped with an outflow tube connected to a trap bottle. A 0.64 cm diameter glass tube was placed in the center of a rubber stopper in the trap bottle and connected to a vacuum pump by rubber tubing. Leachate samples were collected as necessary by replacing the amber glass bottles. Leachate samples from each lysimeter were composited for each 0.5 pore volumes (47.5 L). In addition, background samples were collected from each lysimeter

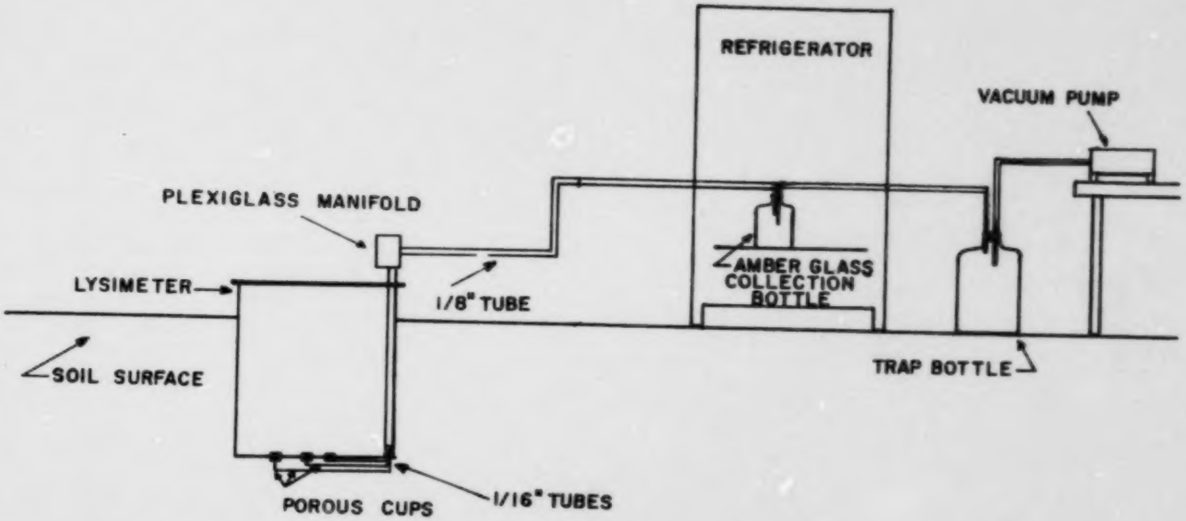
REFRIGERATOR
VACUUM PUMP
PLEXIGLASS MANIFOLD
AMBER GLASS COLLECTION BOTTLE
LYSIMETER
1/8" TUBE
SOIL SURFACE
TRAP BOTTLE
POROUS CUPS
1/16" TUBES

Figure 6-9. Schematic diagram of lysimeters and leachate collection system.

prior to the application of sludge. All collection bottles were cleaned with soap and water and sequentially rinsed with acetone, tap water and distilled water to remove organics prior to sampling. All samples were stored and extracted at 4°C after arrival at the laboratory.

Leachate Water Extraction Procedure

The extraction of leachate water samples was conducted in a walk-in cold room at 4°C. The leachate samples were passed through a glass econo-column 1.5×10^{-2} m x 5×10^{-3} m^2 (Bio-Rad, Richmond, CA) containing a bed of 4×10^{-3} kg or 2×10^{-3} m^2 of XAD-2 resin followed by 6.3×10^{-3} kg or 2×10^{-3} m^2 of XAD-7 resin, as recommended by Rappaport *et al.* (1979). This extraction procedure has been suggested by the Environmental Protection Agency for preparation of environmental samples for mutagenicity (*Salmonella*/microsome) testing (EPA, 1985). The resins were washed prior to use by swirling and decanting three times sequentially with ten volumes each of acetone, methanol, and distilled water. Washed resins were stored at 4°C for no more than 4 weeks prior to use. Dressler (1979) found that the combined XAD-2 and XAD-7 column provided the most efficient recovery for a broad range of organic compounds, while Grabow *et al.* (1981) observed that the XAD technique may be more efficient than a liquid-liquid extraction for the recovery of low concentrations of mutagens.

A glass wool plug was placed above the resin to trap soil particles and prevent the formation of a seal over the resin. Before the leachate water was passed through the column, 1.2 L of distilled water was used to flush the resin. The leachate water collected from the lysimeters was stored in a carboy reservoir above each column and allowed to pass through the column by gravity flow at a rate of approximately 50 mL per min.

After the leachate water collected from each lysimeter was passed through the columns containing the resin, the adsorbed material was extracted as described by Brown and Donnelly (1982). The column was clamped to a ring stand positioned in a chemical fume hood. Dry nitrogen was introduced into the top of the column to remove any residual aqueous phase. The column and glass wool plug were rinsed with 12 L of distilled water to remove any residual histidine which would influence results obtained in the Salmonella/microsome bioassay. The adsorbed organic compounds on the resin and the sediment on the glass wool plug were then eluted with 160 mL of acetone. The acetone extract was filtered through 300 g of anhydrous sodium sulfate and Whatman No. 42 filter paper into a flat bottom flask. The extract was reduced to less than 10 mL on a Brinkman-Bucci Model R Rotary Evaporator at 60°C. The sample was added to a glass funnel filled with sodium sulfate to remove residual water and washed with acetone. The sample was then pipetted into a small Teflon lined screw-capped glass culture tube and taken to dryness under a stream of dry nitrogen. The residue was weighed and diluted with dimethyl sufoxide (Grade 1, Sigma) for testing in the bioassay.

Bioassay

The Salmonella/microsome assay of Ames et al. (1975) was used to evaluate the mutagenicity of the solvent extractable organic compounds in the soil or leachate fractions. The Salmonella strains were kindly supplied by Dr. B.N. Ames (University of California, Berkeley, CA). The procedural methods were the same as Ames et al. (1975) with the modifications suggested by Maron and Ames (1983). The Salmonella tester strain TA98 was utilized with and without metabolic activation because preliminary testing of the sludge fractions indicated that this strain was the most sensitive strain for the types of samples to be evaluated. The S9 mixture (9,000 x g supernatent from homogenized liver) contained 0.3 mL rat-liver S9 and 0.7 mL cofactor supplement (11.4 mM $MgCl_2$, 47 mM KCl, 7.1 mM glucose-6-phosphate, 5.7 mM NADP and 140 mM potassium phosphate buffer at pH 7.4). This high level of metabolic activation was employed in the lysimeter study because preliminary testing of sludge fractions indicated that this was the optimum level for the types of samples anticipated. Aroclor induced Sprague-Dawley rat liver was obtained from Organon Teknika (West Chester, PA). Each of the three fractions obtained from the soil or sludge amended soil was tested in the standard plate incorporation assay at a minimum of five dose levels (mg/plate): 0.5, 1.0, 2.5, 5.0, and 10.0. The data were analyzed using the modified two-fold rule (Chu et al., 1981). Using this procedure, a response is considered positive if the average response for at least two consecutive dose levels was greater than twice the average response for the concurrent negative/solvent control (Chu et al., 1981).

All strains were checked monthly for nutritional markers (histidine and biotin) and other genetic characteristics, i.e., sensitivity to crystal violet, ampicillin, and UV light. On each test date, all tester strains were calibrated with positive and negative controls. Positive controls included 25 µg/plate 2-nitrofluorene (Aldrich, Milwakee, WI); and 2-aminoanthracene (Sigma, St. Louis, MO) at 5 µg/plate was used to verify the functioning of the metabolic activation system. All reagents and extracts were tested for sterility; dimethyl sulfoxide (DMSO, Grade I, Sigma) was used as a solvent control.

Environmental Data

A summary of the environmental data for the duration of the field study is provided in Figure 6-10. The data indicate that following the December application of sludge to six lysimeters, the winter months of 1985-1986 were unseasonably warm, and that the early spring was unusually dry. The maximum soil temperature from January through March ranged from 9.4°C to 17.8°C. The first application to the next twelve lysimeters was accomplished in May, with the second application to the six lysimeters taking place in August. Adequate rainfall was received in April, June, and August; with seasonal rains totalling 26 cm coming in September and October. The winter of 1986-1987 was not as warm as the previous winter, although it was still slightly warmer than normal. The maximum soil temperature from January through March ranged from 8.9°C to 15°C, while the minimum soil temperature ranged from 6.7°C to 13.9°C. The rainfall received in the fall of 1986 and an additional 16.3 cm received in February and March of 1987 assisted in the collection of leachate samples from the lysimeters. The leachate collection system was turned off on April 21, 1987 and the final leachate samples were collected at that time.

RESULTS AND DISCUSSION

Stability

Land application of municipal sludge is designed to transform, degrade,

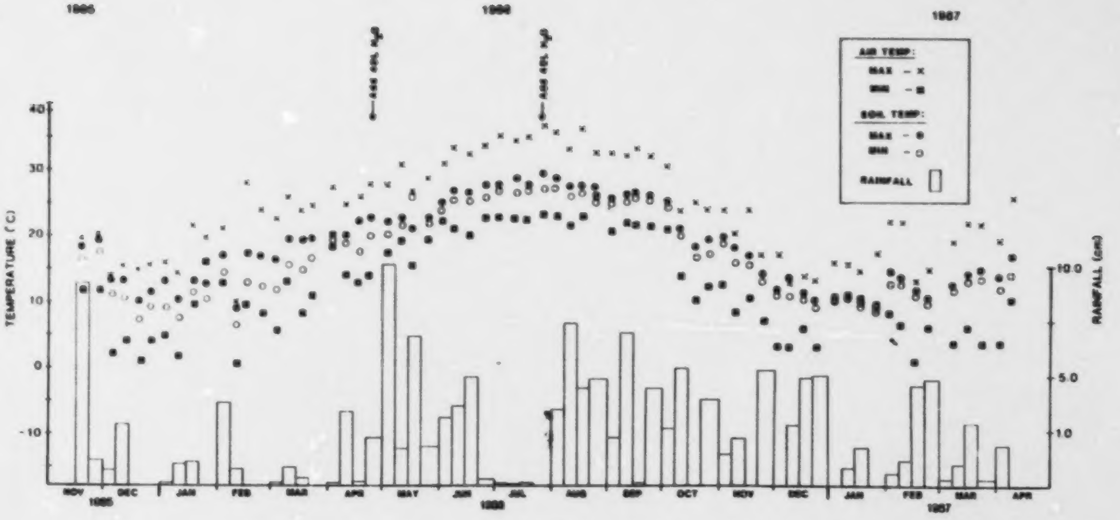

Figure 6-10. Environmental data including rainfall, air temperature and soil temperature for lysimeter field plots for time period during which samples were collected (Note: Data collected at Texas A&M Turf Farm approximately one-half mile northwest of field plots).

or immobilize hazardous constituents through chemical or biological reactions in the soil. The quantity of solvent extractable organics in the sludge amended soil is provided in Table 6-3 and Figure 6-11. These data indicate that the amendment of the Weswood or Padina soils with sewage sludge resulted in a large increase in the amount of solvent extractable organic chemicals in the soil. In the Padina soil which recieved two applications totalling 16.5 kg (150 Mg/ha), the amount of organics recovered from the soil following the first December waste application did not display an obvious decrease until 154 days after waste application. This may be a reflection of slow biodegradation anticipated during the winter months, although the air temperature was unusually warm during this time. The quantity of organics recovered from the soil was reduced from 32 mg/g immediately after waste application, to 20 mg/g and 13 mg/g 154 and 207 days following waste application, respectively (Table 6-3).

The second waste application of 5.5 kg to the Padina soil in August resulted in an increase of solvent extractable organics to 46 mg/g. There was no appreciable decrease in the total extractable organics in the soil collected 54 days after sludge application, although the quantity in the CH_3OH fraction was approximately doubled from immediately after waste application, and the quantity of organics in the CH_2Cl_2 fraction was approximately one-half the value obtained immediately after waste application (Figure 6-11). Similar trends were observed at the lower application rates. At an application rate of 100 Mg/ha (11 kg/lysimeter), the quantity of solvent extractable organics recovered from the Padina sand decreased at 56 days following the May waste application to one-half the

TABLE 6-3. SOLVENT EXTRACTABLE ORGANICS RECOVERED FROM SOILS AMENDED WITH SEWAGE SLUDGE†

Treatment	Fraction	Days After First Application													
		0	3	7	14	28	42	56	116	154	175	207	210	235	263
													Days After Second Application		
													0	28	56
		(Solvent Extractable Organics (mg/g)†													
Padina 150 mT/ha (Dec/Aug)	CH_2Cl_2	11 ± 1	NS	24 ± 19	13 ± 1	15 ± 7	13 ± 6	15 ± 5	NS	9 ± 5	NS	8 ± 2	27 ± 4	21 ± 4	19 ± 2
	CH_3OH	21 ± 1	NS	14 ± 1	14 ± 2	19 ± 1	21 ± 2	15 ± 1	NS	12 ± 2	NS	6 ± 1	19 ± 2	30 ± 3	39 ± 2
	COMB	32 ± 1	NS	39 ± 18	28 ± 3	34 ± 7	52 ±18	30 ± 7	NS	20 ± 3	NS	13 ± 3	46 ± 2	52 ± 2	57 ± 3
Padina 100 mT/ha (May)	CH_2Cl_2	19 ± 5	NS	NS	NS	13 ± 6	NS	8 ± 3	8 ± 2	NS	9 ± 0	NS	NS	NS	NS
	CH_3OH	17 ± 6	NS	NS	NS	13 ± 7	NS	5 ± 1	7 ± 3	NS	8 ± 2	NS	NS	NS	NS
	COMB	37 ± 11	NS	NS	NS	27 ±12	NS	13 ± 3	15 ± 5	NS	17 ± 2	NS	NS	NS	NS
Padina 50 mT/ha (May)	CH_2Cl_2	9 ± 2	NS	8 ± 3	NS	4 ± 1	NS	3 ± 1	4 ± 1	NS	3 ± 1	NS	NS	NS	NS
	CH_3OH	7 ± 0	NS	9 ± 2	NS	9 ± 6	NS	3 ± 1	3 ± 1	NS	2 ± 0	NS	NS	NS	NS
	COMB	16 ± 2	NS	15 ± 5	NS	8 ± 6	NS	7 ± 2	7 ± 1	NS	5 ± 2	NS	NS	NS	NS
Weswood 150 mT/ha (Dec/Aug)	CH_2Cl_2	11 ± 3	18 ± 9	25 ± 8	19 ± 4	15 ± 4	16 ± 2	12 ± 0	NS	9 ± 2	NS	5 ± 1	29 ± 2	18 ± 4	20 ± 2
	CH_3OH	37 ± 11	20 ± 2	24 ± 4	24 ± 1	20 ± 3	18 ± 4	14 ± 1	NS	8 ± 1	NS	8 ± 2	18 ± 2	20 ± 15	26 ± 13
	COMB	48 ± 12	39 ± 3	49 ± 11	43 ± 4	35 ± 4	33 ± 2	25 ± 0	NS	17 ± 1	NS	13 ± 3	47 ± 2	37 ± 14	46 ± 8
Weswood 100 mT/ha (May)	CH_2Cl_2	13 ± 3	NS	13 ± 3	6 ± 1	8 ± 1	NS	5 ± 0	5 ± 0	NS	5 ± 0	NS	NS	NS	NS
	CH_3OH	11 ± 2	NS	12 ± 3	5 ± 1	9 ± 1	NS	5 ± 1	4 ± 1	NS	4 ± 1	NS	NS	NS	NS
	COMB	24 ± 4	NS	24 ± 4	11 ± 1	15 ± 1	NS	11 ± 1	9 ± 0	NS	10 ± 1	NS	NS	NS	NS
Weswood 50 mT/ha (May)	CH_2Cl_2	13 ± 2	NS	12 ± 1	6 ± 1	6 ± 1	NS	4 ± 1	6 ± 2	NS	4 ± 1	NS	NS	NS	NS
	CH_3OH	9 ± 2	NS	9 ± 2	5 ± 1	4 ± 1	NS	5 ± 2	4 ± 0	NS	2 ± 1	NS	NS	NS	NS
	COMB	22 ± 4	NS	21 ± 3	11 ± 3	8 ± 3	NS	9 ± 2	10 ± [illegible]	NS	6 ± 1	NS	NS	NS	NS
Padina Control	CH_2Cl_2	0.57 ± .3	NS	NS	0.39± .2	NS	NS	0.15± .08	NS	NS					
	CH_3OH	0.46 ± .3	NS	NS	0.59± .4	NS	NS	0.44± .07	NS	NS					
	COMB	1.03 ± .6	NS	NS	0.98± .1	NS	NS	0.59± .15	NS	NS					
Weswood Control	CH_2Cl_2	0.16 ± .06	NS	NS	0.40± .5	NS	NS	0.21± .05	NS	NS					
	CH_3OH	1.31 ± .11	NS	NS	0.60± .3	NS	NS	0.81± .51	NS	NS					
	COMB	1.48 ± .09	NS	NS	1.00± .2	NS	NS	1.03± .47	NS	NS					

† NS = not sampled; CH_2Cl_2 = methylene chloride; CH_3OH = methanol; COMB = combined; † Mean ± Standard Deviation.

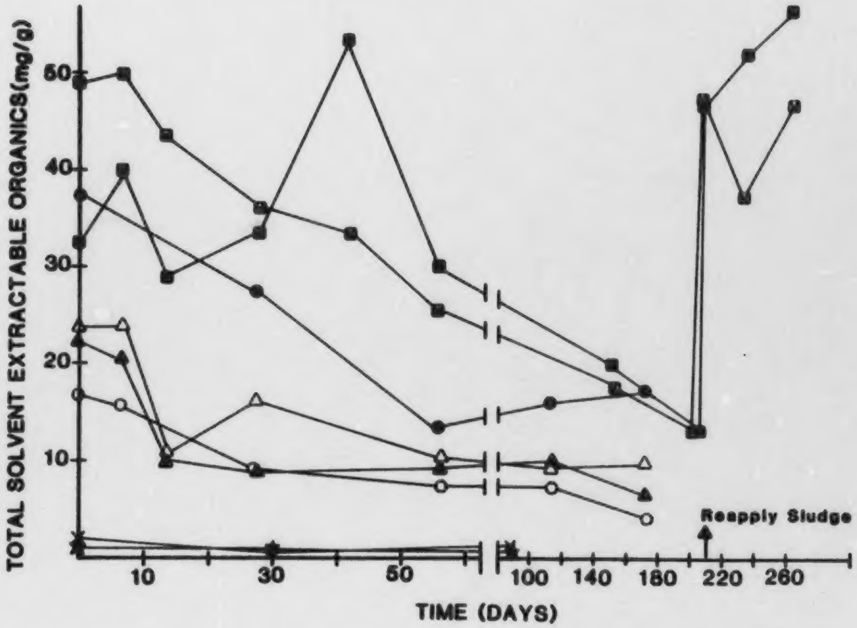

Figure 6-11. Total solvent extractable organics (methylene chloride plus methanol) recovered from sludge amended and control lysimeters. Legend: Padina sand receiving 150 Mg/ha total sludge applied (■), Padina sand receiving one 100 Mg/ha sludge application (●), Padina sand receiving one 50 Mg/ha sludge application (O), Padina sand control (★), Weswood loam receiving 150 Mg/ha total sludge applied (⊠), Weswood loam receiving one 100 Mg/ha sludge application (▲), Weswood loam receiving one 50 Mg/ha sludge application (△), Weswood loam control (X).

amount recovered immediately after waste application. This amount was, however, very stable and approximately the same quantity was recovered 175 days after waste application. At the lowest application rate (5.5 kg/lysimeter or 50 Mg/ha), the solvent extractable organics obtained from the Padina sand decreased from 16 mg/g immediately after waste application to 7 mg/g 56 days after the May waste application (Table 6-3). The quantity of organics recovered from the low application rate remained stable, as 5 mg/g were obtained from the sample collected 175 days after the May application (Table 6-3).

The quantity of solvent extractable organics in the sludge amended Weswood soil was also observed to decrease very slowly over time (Figure 6-11. The results presented in Table 6-3 indicate that for the December application of 150 Mg/ha, the amount of solvent extractable organics recovered from the waste amended soil was appreciable reduced from 48 mg/g immediately after waste application to 25 mg/g by 56 days after waste application; and, was further reduced to 13 mg/g 207 days after waste application. As was observed with the Padina soil, the August application of 5.5 kg/lysimeter increased the solvent extractable organics appreciably, and did not result in a decrease throughout the 56 days following the second waste application.

For the May application of 100 Mg/ha, the quantity of solvent extractable organics was reduced to approximately one-half the initial value 56 days following waste application (Figure 6-11). However, this amount was stable, and the quantity of solvent extractable organics recovered from the soil was not appreciably decreased in the sample collected 175 days after waste application. At the lowest application rate to the Weswood soil (50 Mg/ha), the quantity of solvent extractable organics was appreciably reduced by the 28th day following the May application; however, this amount was not further reduced in the sample collected 175 days after waste application (Figure 6-11). It should be noted that the two application rates to the Weswood soil resulted in essentially equal amounts of organics extracted from the soil initially, although the level in the high application was more slowly reduced than the organics in the low application rate. This may indicate that there was some binding in the Weswood soil at the highest application rate, suggesting that solvent extraction could not completely recover the bound organics from the Weswood soil.

For all soils and all application rates, the quantity of solvent extractable organics in the sludge amended soil remained at least five times the quantity in the unamended soil more than 175 days after waste application. For the Padina sand, the approximate half lives for the total solvent extractable organics in the sludge amended soil ranged from a low of 109 days for the low application rate in May, to 168 days for the December application of 100 Mg/ha. For the Weswood soil, the approximate half lives ranged from 95 days for the low application rate in May, to 146 for the high application rate in May. In addition, none of the soils which received two waste applications displayed sufficient loss of solvent extractable organics following the second sludge application to calculate a half life. Thus, the approximate range for half lives of organics in the sludge amended soil was from 95 to 168 days.

The specific activity of the solvent extractable organics recovered from the sludge amended soil will provide a qualitative indication of the mutagenic potential of the sludge organics. The values presented in Table 6-4 represents the net TA98 his^+ revertants per plate when tested at a dose level of 10 mg per plate. The results presented in Figures 6-12 and 6-13 display the specific activity of the soil from the Padina sand lysimeters which received two sludge applications totalling 150 Mg/ha. In the absence of metabolic activation, the CH_2Cl_2 fraction of the sludge and initial sludge amended soil sample induced 119 and 140 net revertants, respectively (Tables 6-1 and 6-4). Following the first application of 100 Mg/ha of sludge to the Padina sand, the mutagenic potential without metabolic activation of the CH_2Cl_2 extractable organics was reduced by approximately 50% on the 7th day following waste application (Figure 6-12). This reduction was followed by an increase on day 14 and 28 to a level which remained constant over the remaining time prior to reapplication. The maximum response without metabolic activation for the CH_2Cl_2 fraction of the sludge amended Padina sand was 164 net revertants observed in the sample collected 28 days after waste application. The direct-acting CH_2Cl_2 extractable organics appears to be quite stable, as the mutagenic potential was 140 immediately following waste application, and 116 net revertants 207 days following waste application (Table 6-4). The application of 5.5 kg of sludge (50 Mg/ha) in August reduced the mutagenic potential without activation of the CH_2Cl_2 fraction of the soil to a level which would be considered negative for all samples collected during the 56 days following sludge application (Table 6-4).

TABLE 6-4. SPECIFIC ACTIVITY, AS MEASURED WITH S. *typhimurium* STRAIN TA98 OF SOLVENT EXTRACTABLE ORGANICS RECOVERED FROM SOILS †

Treatment	Fraction	Days After First Application									
		0		7		14		28		42	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Padina	CH_2Cl_2	140 ± 35	74 ± 7	86 ± 11	88 ± 15	112 ± 41	91 ± 9	164 ± 42	93 ± 24	143 ± 13	84 ± 22
150 Mg/ha	CH_3OH	50 ± 12	70 ± 21	59 ± 30	129 ± 37	43 ± 8	139 ± 22	46 ± 8	195 ± 28	37 ± 2	210 ± 15
(Dec/Aug)	COMB	97 ± 25	116 ± 37	55 ± 10	158 ± 8	77 ± 17	179 ± 77	90 ± 9	171 ± 27	56 ± 8	137 ± 34
Padina	CH_2Cl_2	137 ± 24	55 ± 11	NS	NS	NS	NS	NS	NS	NS	NS
100 Mg/ha	CH_3OH	78 ± 29	44 ± 8	NS	NS	NS	NS	NS	NS	NS	NS
(May)	COMB	109 ± 22	50 ± 7	NS	NS	NS	NS	NS	NS	NS	NS
Padina	CH_2Cl_2	122 ± 8	79 ± 22	116 ± 43	103 ± 17	NS	NS	NS	NS	NS	NS
50 Mg/ha	CH_3OH	61 ± 15	41 ± 15	43 ± 23	90 ± 15	NS	NS	NS	NS	NS	NS
(May)	COMB	111 ± 35	49 ± 21	94 ± 31	102 ± 14	NS	NS	NS	NS	NS	NS
Weswood	CH_2Cl_2	111 ± 34	79 ± 3	71 ± 16	83 ± 11	137 ± 17	90 ± 2	143 ± 6	88 ± 8	77 ± 11	116 ± 8
150 Mg/ha	CH_3OH	46 ± 9	115 ± 38	29 ± 2	113 ± 36	37 ± 3	135 ± 81	43 ± 7	221 ± 48	32 ± 3	235 ± 44
(Dec/Aug)	COMB	88 ± 15	106 ± 30	44 ± 16	119 ± 13	69 ± 9	119 ± 29	67 ± 21	175 ± 25	46 ± 13	216 ± 45
Weswood	CH_2Cl_2	184 ± 14	60 ± 22	158 ± 16	84 ± 37	NS	NS	NS	NS	NS	NS
100 Mg/ha	CH_3OH	76 ± 18	53 ± 12	42 ± 15	68 ± 28	NS	NS	NS	NS	NS	NS
(May)	COMB	147 ± 10	70 ± 9	76 ± 17	83 ± 15	NS	NS	NS	NS	NS	NS
Weswood	CH_2Cl_2	167 ± 46	75 ± 10	142 ± 24	88 ± 11	NS	NS	NS	NS	NS	NS
50 Mg/ha	CH_3OH	65 ± 15	47 ± 9	51 ± 17	76 ± 11	NS	NS	NS	NS	NS	NS
(May)	COMB	111 ± 15 ††	60 ± 9 ††	100 ± 19	48 ± 9	NS	NS	NS	NS	NS	NS
Padina	CH_2Cl_2	13 ± 15	10 ± 9	NS	NS	NS	11 ± 4	8 ± 5	NS	NS	NS
Control	CH_3OH	27 ± 24	5 ± 4	NS	NS	NS	76 ± 1	27 ± 13	NS	NS	NS
	COMB	10 ± 9	3 ± 4	NS	NS	NS	59 ± 12	19 ± 1	NS	NS	NS
Weswood	CH_2Cl_2	11 ± 9	11 ± 11	NS	NS	NS	12 ± 3	8 ± 5	NS	NS	NS
Control	CH_3OH	32 ± 28	11 ± 8	NS	NS	NS	47 ± 4	18 ± 5	NS	NS	NS
	COMB	13 ± 6	9 ± 12	NS	NS	NS	41 ± 11	17 ± 2	NS	NS	NS

TABLE 6-4 CONTINUED

Treatment	Fraction	Days After First Application									
		56		116		154		175		207	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Padina	CH_2Cl_2	128 ± 22	115 ± 36	NS	NS	128 ± 10	226 ± 31	NS	NS	116 ± 19	150 ± 12
150 Mg/ha	CH_3OH	31 ± 5	219 ± 14	NS	NS	38 ± 10	320 ± 56	NS	NS	32 ± 7	317 ± 32
(Dec/Aug)	COMB	80 ± 24	196 ± 22	NS	NS	45 ± 7	265 ± 23	NS	NS	90 ± 18	247 ± 22
Padina	CH_2Cl_2	101 ± 25	169 ± 17	42 ± 16	111 ± 21	NS	NS	40 ± 6 ††	86 ± 13††	NS	NS
100 Mg/ha	CH_3OH	68 ± 30	139 ± 8	36 ± 5	100 ± 14	NS	NS	33 ± 4 ††	88 ± 8††	NS	NS
(May)	COMB	111 ± 39	168 ± 28	40 ± 2	118 ± 11	NS	NS	30 ± 11 ††	75 ± 4††	NS	NS
Padina	CH_2Cl_2	129 ± 37	141 ± 53	102 ± 45	111 ± 29	NS	NS	56 ± 20	73 ± 9	NS	NS
50 Mg/ha	CH_3OH	156 ± 94	154 ± 47	54 ± 21	59 ± 25	NS	NS	73 ± 20	117 ± 21	NS	NS
(May)	COMB	132 ± 56	136 ± 47	33 ± 9	69 ± 33	NS	NS	69 ± 17	101 ± 14	NS	NS
Weswood	CH_2Cl_2	161 ± 32	133 ± 41	NS	NS	131 ± 22	297 ± 19	NS	NS	109 ± 32	94 ± 15
150 Mg/ha	CH_3OH	40 ± 7	211 ± 38	NS	NS	45 ± 10	416 ± 73	NS	NS	163 ± 56	269 ± 28
(Dec/Aug)	COMB	102 ± 5	182 ± 28	NS	NS	81 ± 23	333 ± 44	NS	NS	87 ± 46	96 ± 59
Weswood	CH_2Cl_2	144 ± 55	159 ± 36	105 ± 49	149 ± 37	NS	NS	53 ± 19	100 ± 22	NS	NS
100 Mg/ha	CH_3OH	202 ± 29	181 ± 24	183 ± 71	194 ± 41	NS	NS	42 ± 37 ††	64 ± 53††	NS	NS
(May)	COMB	180 ± 32	150 ± 34	174 ± 35	194 ± 43	NS	NS	54 ± 35 ††	49 ± 39 ††	NS	NS
Weswood	CH_2Cl_2	186 ± 45	174 ± 17	58 ± 49 ††	98 ± 11 ††	NS	NS	132 ± 46	172 ± 50	NS	NS
50 Mg/ha	CH_3OH	222 ± 37	169 ± 31	107 ± 5 ††	83 ± 25 ††	NS	NS	72 ± 46 ††	46 ± 13 ††	NS	NS
(May)	COMB	200 ± 27	171 ± 6	139 ± 7 ††	116 ± 13 ††	NS	NS	88 ± 12 ††	59 ± 7 ††	NS	NS
Padina	CH_2Cl_2	20 ± 29	11 ± 20	NS	NS	NS	NS	NS	NS	NS	NS
Control	CH_3OH	48 ± 5	6 ± 3	NS	NS	NS	NS	NS	NS	NS	NS
	COMB	23 ± 4	2 ± 4	NS	NS	NS	NS	NS	NS	NS	NS
Weswood	CH_2Cl_2	8 ± 7	6 ± 10	NS	NS	NS	NS	NS	NS	NS	NS
Control	CH_3OH	43 ± 4	7 ± 8	NS	NS	NS	NS	NS	NS	NS	NS
	COMB	15 ± 8	6 ± 10	NS	NS	NS	NS	NS	NS	NS	NS

TABLE 6-4 CONTINUED

Treatment	Fraction	Days After First Application 210 Days After Second Application 0 -S9	+S9	235 28 -S9	+S9	263 56 -S9	+S9
Padina 150 mT/ha (Dec/Aug)	CH_2Cl_2	46 ± 11	79 ± 21	NS	NS	35 ± 4	108 ± 26
	CH_3OH	19 ± 7	93 ± 22	29 ± 3	163 ± 15	29 ± 3	163 ± 15
	COMB	50 ± 22	118 ± 29	36 ± 5	136 ± 13	36 ± 5	136 ± 13
Padina 100 mT/ha (May)	CH_2Cl_2	NS	NS	NS	NS	NS	NS
	CH_3OH	NS	NS	NS	NS	NS	NS
	COMB	NS	NS	NS	NS	NS	NS
Padina 50 mT/ha (May)	CH_2Cl_2	NS	NS	NS	NS	NS	NS
	CH_3OH	NS	NS	NS	NS	NS	NS
	COMB	NS	NS	NS	NS	NS	NS
Weswood 150 mT/ha (Dec/Aug)	CH_2Cl_2	113 ± 23	87 ± 13	47 ± 16	197 ± 13	74 ± 8	169 ± 13
	CH_3OH	45 ± 6	131 ± 26	34 ± 4	188 ± 15	57 ± 11	177 ± 9
	COMB	70 ± 10	102 ± 22	45 ± 15	208 ± 31	72 ± 14	179 ± 21
Weswood 100 mT/ha (May)	CH_2Cl_2	NS	NS	NS	NS	NS	NS
	CH_3OH	NS	NS	NS	NS	NS	NS
	COMB	NS	NS	NS	NS	NS	NS
Weswood 50 mT/ha (May)	CH_2Cl_2	NS	NS	NS	NS	NS	NS
	CH_3OH	NS	NS	NS	NS	NS	NS
	COMB	NS	NS	NS	NS	NS	NS
Padina Control	CH_2Cl_2	NS	NS	NS	NS	NS	NS
	CH_3OH	NS	NS	NS	NS	NS	NS
	COMB	NS	NS	NS	NS	NS	NS
Weswood Control	CH_2Cl_2	NS	NS	NS	NS	NS	NS
	CH_3OH	NS	NS	NS	NS	NS	NS
	COMB	NS	NS	NS	NS	NS	NS

[†] NS = not sampled; CH_2Cl_2 = methylene chloride; CH_3OH = methanol; COMB = combined. Each data point represents the mean net revertant (± SD) for three samples tested on duplicate plates in two independent experiments (N=12) except where noted. DMSO control values for study were 28 revertants without metabolic activation and 36 revertants with activation.†† Data represents mean net revertants (± SD) for two samples tested on duplicate plates in two indpendent experiments (N=8).

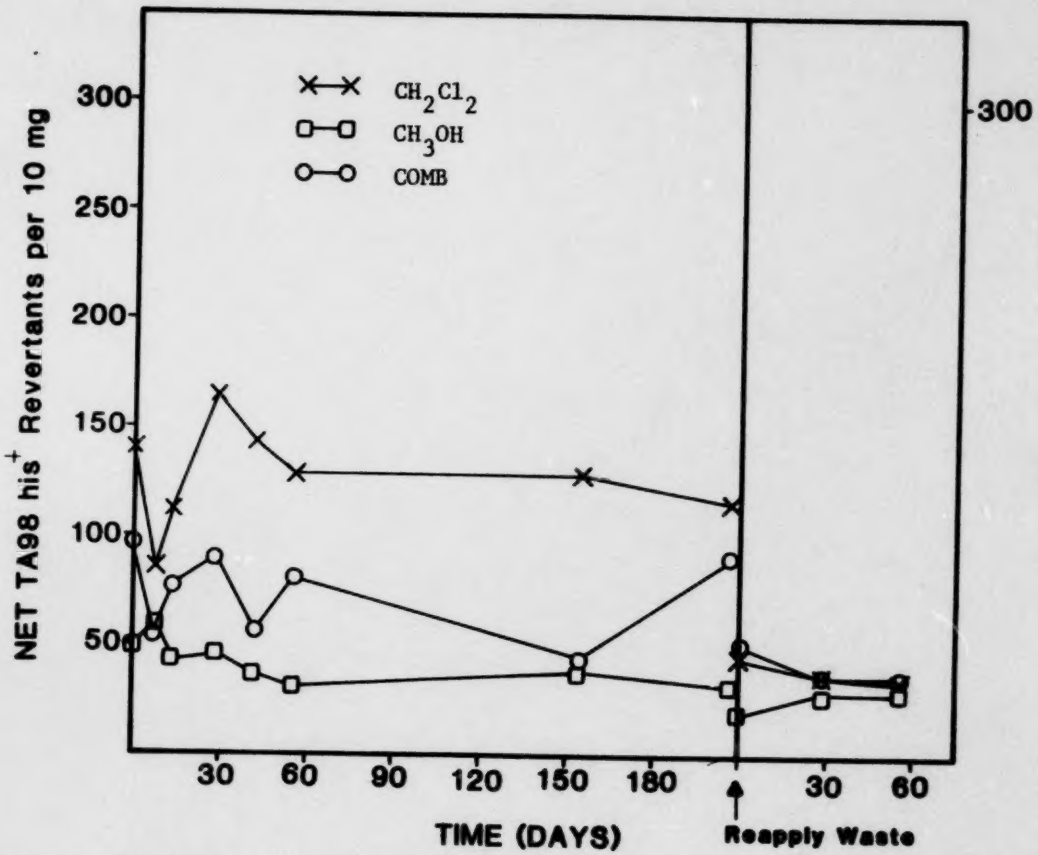

Figure 6-12. Bacterial mutagenicity, as measured without metabolic activation of methylene chloride (CH_2Cl_2), methanol (CH_3OH) and combined fractions (COMB) extracted from Padina sand lysimeters which received two sludge applications totalling 150 Mg/ha.

With metabolic activation, the mutagenic potential of the CH_2Cl_2 fraction collected from the waste amended Padina sand was generally reduced from the level observed without metabolic activation. However, for the samples collected 154 and 207 days following waste application, the addition of metabolic activation to the assay system increased the mutagenic potential of the CH_2Cl_2 fraction. With metabolic activation, the CH_2Cl_2 fraction extracted from the SM3 sludge induced 25 net revertants (Table 6-1); while, the CH_2Cl_2 fraction extracted from the Padina soil induced 74 net revertants immediately following waste application, and 226 net revertants 154 days following waste application (Figure 6-13). In addition, following the second waste application to the Padina sand, the mutagenic potential of the CH_2Cl_2 fraction was enhanced by the addition of metabolic activation. The CH_2Cl_2 fraction of the Padina sand collected 56 days after the second sludge application induced 35 net revertants without metabolic activation, and 108 net revertants with activation (Table 6-4). Similarly, the sludge applied to the Padina sand in August contained CH_2Cl_2 soluble organics which induced a stronger mutagenic response with methabolic activation than without (Table 6-1).

The same general trend was observed for the specific activity of the CH_3OH fraction from the sludge amended Padina sand which received two sludge applications totalling 16.5 kg (150 Mg/ha), although the response at each sample date was slightly lower than was observed for the CH_2Cl_2 fraction. The CH_3OH fraction failed to induce a positive response without activation at any of the dates samples were collected. The addition of metabolic activation to the assay system generally increased the mutagenic

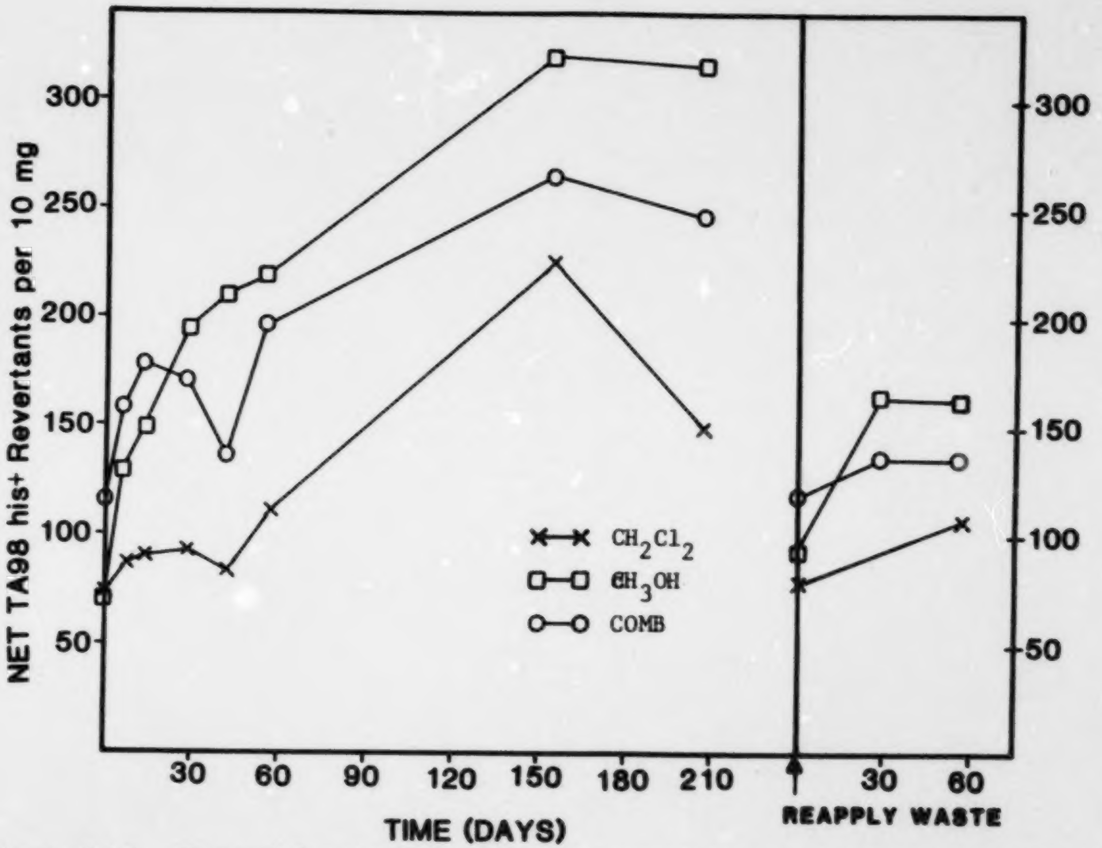

Figure 6-13. Bacterial mutagenicity, as measured with metabolic activation of methylene chloride (CH_2Cl_2), methanol (CH_3OH) and combined fractions (COMB) extracted from Padina sand lysimeters which received two sludge applications totalling 150 Mg/ha.

potential of the CH_2OH fraction extracted from the sludge amended soil, as was observed for the CH_3OH fraction extracted from the sludge (Table 6-1 and 6-4). The mutagenic potential of the methanol CH_3OH extracted from the sludge and the initial sample extracted from the sludge amended soil in the presence of metabolic activation was 87 and 70 net revertants, respectively (Tables 6-1 and 6-4). With metabolic activation, the mutagenic potential of the CH_3OH fraction was observed to increase through 154 days following sludge application at which time the CH_3OH fraction induced a response of 320 net revertants which was approximately four times greater than the response induced by the sample collected immediately after waste application (Figure 6-13). The response was reduced slightly in the sample collected 207 days after application, and the application of 5.5 kg sludge in August further reduced the mutagenic response to a level approximately equal to the level observed in the sample collected immediately after the first waste application (Figure 6-13). However, the mutagenic potential of the CH_3OH fraction increased appreciably from 93 net revertants immediately following the second waste application to 163 net revertants in the sample collected 28 days after the second waste application, and remained constant in the sample collected 56 days after waste application (Table 6-4).

The combined fraction extracted from both the sludge and the Padina sand which received two sludge applications induced a positive response with and without metabolic activation. In the absence of metabolic activation, the combined fraction extracted from the sludge amended soil collected immediately after waste application induced 97 net revertants. In the presence of metabolic activation, the response was 116 net revertants (Table 6-4). The combined fraction extracted from the sludge induced 80 and 71 net revertants without and with metabolic activation, respectively (Table 6-1). Although the combined fraction extracted from the sludge amended soil 154 days following waste application induced only 45 net revertants without metabolic activation, the majority of the samples collected induced a response that was not appreciably different from the response observed in the sample collected immediately after waste application (Figure 6-12). With metabolic activation, the maximum response was observed in the sample collected 154 days after waste application (Figure 6-13). After the second application of sludge to the Padina sand lysimeters, the response without metabolic activation remained at a level only slightly greater than twice background for the entire 56 days samples were collected (Figure 6-12). In the presence of metabolic activation, the mutagenic potential of the combined fraction increased from 63 net revertants in the sludge (Table 6-1), to 118 net revertants in the combined fraction extraced from the sample collected immediately following waste application, to a maximum of 136 net revertants in the samples collected 28 and 56 days following waste application (Table 6-4).

The specific activity of the combined fraction can be used to describe the interactions of the components of the two individual fractions. Without metabolic activation, the response observed from the combined fraction appears to indicate that the CH_3OH fraction is inhibiting the mutagenicity of the CH_2Cl_2 fraction. With metabolic activation, the combined fraction appears to represent the additive mutagenicity of the two fractions for the samples collected from immediately following waste application through 14 days following waste application (Table 6-4). For all samples collected following the 14th day after waste application, the mutagenicity of the combined fraction was reduced when compared to the CH_3OH fraction. Thus, the two fractions when combined induced additive responses until the CH_3OH fraction displayed a large increase in its mutagenic potential, at which time the CH_2Cl_2 fraction inhibited the indirect acting mutagenicity of the CH_3OH fraction. The specific activity with activation of the combined fraction from the Padina soil which received 150 Mg/ha of sludge ranged from 116 net revertants immediately following sludge application, to 265 net revertants for the combined fraction collected 154 days after waste application (Table 6-4).

The results presented in Table 6-4 and Figure 6-14 describe the specific activity of the solvent extractable organics for the Padina sand which received one sludge application of 11kg (100 Mg/ha) in May, 1986. These data indicate that the specific activity without activation of the CH_2Cl_2 fraction of the sludge amended soil was appreciably reduced in the sample collected 116 days following waste application and remained at a level which was slightly greater than twice the solvent control in the sample collected 175 days after waste application (Figure 6-14). Without metabolic activation, the CH_2Cl_2 fraction extracted from the SM4 sludge induced 122 net revertants (Table 6-1), while the sample collected immediately after waste application induced 137 net revertants, and the samples collected 116 and 175 days after waste application induced 42 net revertants and 40 net revertants, respectively (Table 6-4). With metabolic activation, the CH_2Cl_2 fraction of the SM4 sludge induced 21 net revertants

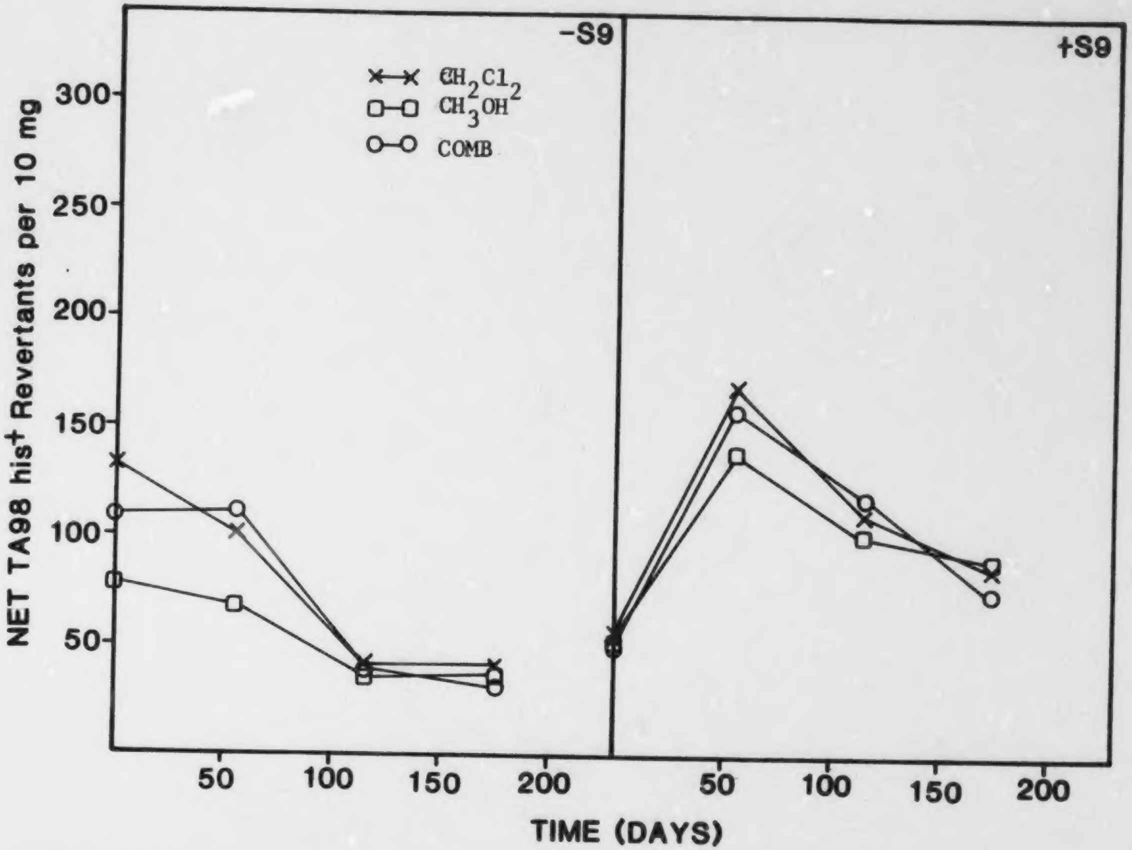

Figure 6-14. Bacterial mutagenicity, as measured with and without metabolic activation of methylene chloride (CH_2Cl_2), methanol (CH_3OH) and combined fractions (COMB) extracted from Padina sand lysimeters which received one sludge application of 100 Mg/ha.

(Table 6-1), while the sample collected immediately after waste application induced 55 net revertants, the sample collected 116 days after waste application induced 111 net revertants and the sample collected 175 days after waste application induced 86 net revertants (Table 6-4). These data indicate that over time there may have been a shift in the mutagenic characteristics of the CH_2Cl_2 fraction from direct acting mutagens to indirect acting mutagens.

For the CH_3OH fraction extracted from the Padina sand which received one sludge application of 100 Mg/ha (100 Mg/ha) in May, significant levels of mutagenic activity were also observed. Although the CH_3OH fraction of the sludge applied in May did not induce a positive response with or without metabolic activation (Table 6-1), the CH_3OH fraction from the sludge amended soil did induce a positive response (Table 6-4). Without metabolic activation the mutagenic potential of the CH_3OH fraction was decreased at each sampling date, while the mutagenic potential with activation reached a maximum in the sample collected 56 days after waste application and was reduced approximately 30% in the sample collected 116 days after waste application (Figure 6-14). In the absence of metabolic activation, the CH_3OH fraction extracted from the Padina sand induced 78 net revertants immediately following waste application, while the sample collected 175 days following waste application induced 33 net revertants (Table 6-4). With metabolic activation, the CH_3OH fraction collected immediately following waste application induced 44 net revertants, the sample collected 56 days after waste application induced 139 net revertants, and the sample collected 175 days after waste application induced 88 net revertants (Table 6-4).

The combined fraction extracted from the Padina sand which received one application of 100 Mg/ha of sludge in May displayed a large degree of variability in its mutagenic potential. The mutagenic potential of the combined fraction most closely approximated the mutagenic potential of the CH_2Cl_2 fraction with and without metabolic activation (Figure 6-14). With and without metabolic activation, the mutagenic potential of the combined fraction reached a maximum 56 days after waste application and was appreciably reduced in the sample collected 116 days after waste application (Figure 6-14). The specific activity without metabolic activation of the combined fraction extracted from the SM4 sludge induced 95 net revertants (Table 6-1), while the combined fraction extracted from the sludge amended soil induced 109, 111, 40 and 30 net revertants immediately following, 56, 116 and 175 days following waste application, respectively (Table 6-4). With metabolic activation, the combined fraction from the sludge induced 28 net revertants (Table 6-1), while the combined fraction from the sludge amended Padina sand induced 50, 168, 118 and 75 net revertants immediately following, 56, 116, and 175 days after waste application (Table 6-4). In general the mutagenic potential of the combined fraction was approximately equal to the activity of the corresponding CH_2Cl_2 fraction.

At the lowest sludge application rate of 50 Mg/ha to the Padina sand, the specific activity of the CH_2Cl_2 fraction without metabolic activation was essentially unchanged until 175 days following sludge application (Figure 6-15). The specific activity without activation of the CH_2Cl_2

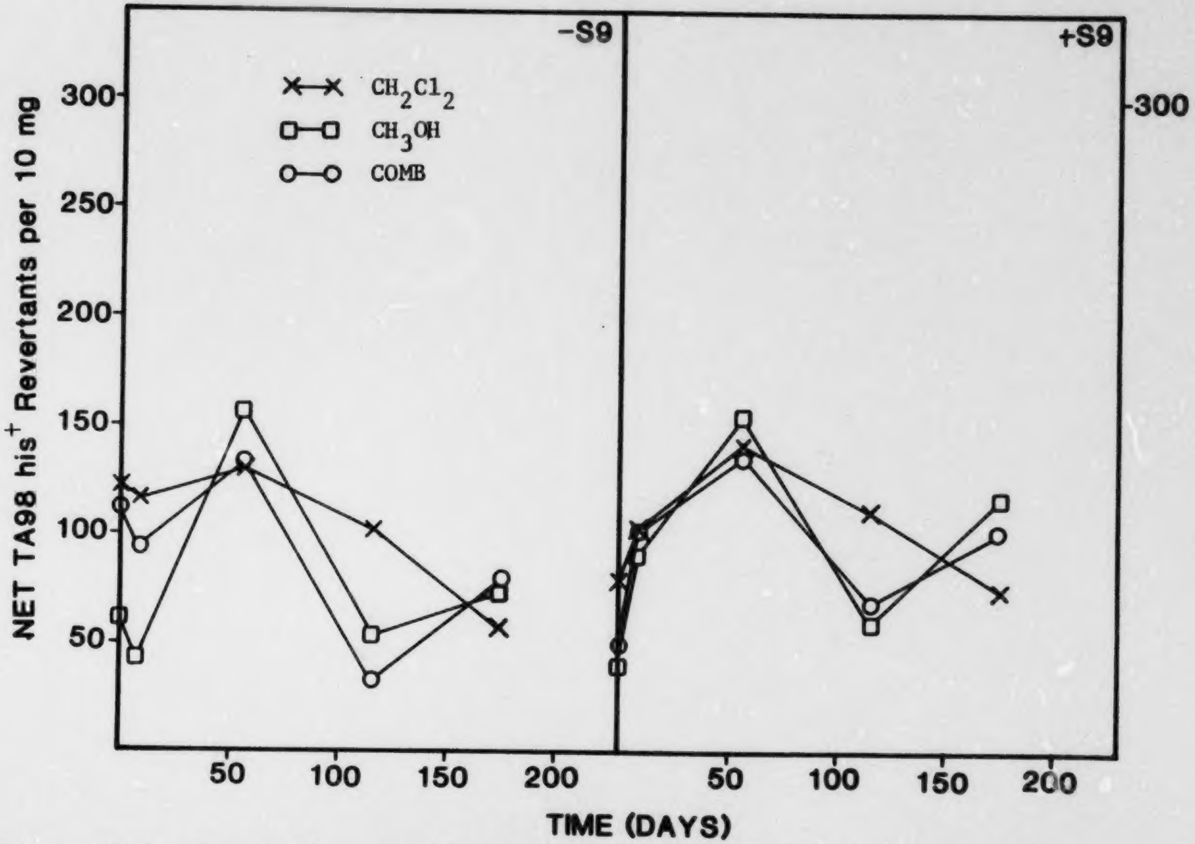

Figure 6-15. Bacterial mutagenicity, as measured with and without metabolic activation, of methylene chloride (CH_2Cl_2), methanol (CH_3OH), and combined fractions (COMB) extracted from Padina sand lysimeters which received one sludge application of 50 Mg/ha.

fraction extracted from the SM4 sludge was 122 net revertants (Table 6-1), while the mutagenic potential of the CH_2Cl_2 fraction collected immediately following, 116 and 175 days after waste application was 122, 102, and 56 net revertants, respectively (Table 6-4). With metabolic activation, the specific activity of the CH_2Cl_2 fraction extracted from the SM4 sludge was 21 net revertants (Table 6-1), while the CH_2Cl_2 fraction extracted from the sludge amended soil collected 56 days after waste application induced 141 net revertants, and the sample collected 175 days after waste application induced 73 net revertants (Table 6-4).

The specific activity of the CH_3OH extract from the Padina sand which received one application of 5.5 kg of sludge (50 Mg/ha) was relatively unchanged until 56 days after waste application (Figure 6-15). Without metabolic activation, the mutagenic potential of the CH_3OH fraction was 61 net revertants immediately following waste application, and 156 net revertants 56 days after waste application (Table 6-4). Without activation, this response was reduced in the samples collected 116 and 175 days after waste application (Figure 6-15) to a level approximately equal to that observed in the sample collected immediately after waste application. With metabolic activation, the CH_3OH fraction of the Padina sand which received one application of 50 Mg/ha of sludge induced 41 net revertants immediately after waste application, and 90 net revertants 7 days after waste application (Table 6-4). This response increased to 154 net revertants in the sample collected 56 days after waste application, but was reduced to 117 net revertants in the sample collected 175 days after waste application (Table 6-4).

Without activation, the specific activity of the combined fraction extracted from the sludge amended Padina soil which received 50 Mg/ha of SM4 sludge in May was 111 net revertants immediately following waste application and 132 net revertants 56 days after application (Table 6-4). This response was reduced to 33 net revertants or a level which would be considered non-mutagenic in the combined fraction collected 116 days following waste application (Table 6-4). In the presence of metabolic activation, the mutagenic potential of the combined fraction extracted from the Padina sand was approximately doubled in the sample collected 7 days after waste application, and remained constant through the 175th day after waste application (Figure 6-15). The specific activity of the combined fraction extracted from the Padina soil 175 days after sludge application was 69 and 101 net revertants without and with metabolic activation, respectively (Table 6-4). The response observed in the combined fraction generally paralleled the corresponding response observed in the CH_3OH fraction with and without metabolic activation (Figure 6-15).

The specific activity of the Weswood soil which received two sludge applications totalling 16.5 kg (150 Mg/ha) followed a similar trend to the Padina soil for both direct and indirect-acting mutagens. The CH_2Cl_2 fraction extracted from the SM4 sludge applied to the Weswood soil induced 122 and 21 net revertants without and with metabolic activation, respectively (Table 6-1). The mutagenic potential without metabolic activation of the CH_2Cl_2 fraction extracted from the sludge amended Weswood soil was highly variable during the first 56 days samples were collected and relatively unchanged in the sample collected 207 days after sludge application (Figure 6-16). In the absence of metabolic activation, the specific activity of CH_2Cl_2 fraction extracted from the sludge amended soil was 111 net revertants immediately following sludge application, 161 net revertants 56 days after application, and 109 net revertants 207 days after application (Table 6-4). Following the second sludge application to the Weswood soil, the specific activity of the CH_2Cl_2 fraction without activation was 113 net revertants immediately after, and 74 net revertants 56 days after the second sludge application (Table 6-4).

The indirect-acting mutagenicity of the CH_2Cl_2 fraction extracted from the Weswood soil increased dramatically 156 days after sludge application and was reduced by more than 60% in the sample collected 207 days after sludge application (Figure 6-17). With metabolic activation, the CH_2Cl_2 fraction extracted from the sludge amended Weswood soil induced 79 net revertants immediately following waste application, 297 net revertants 154 days after application, and 94 net revertants 207 days after application (Table 6-4). After the second sludge application, the mutagenic potential with activation was 87 net revertants immediately following waste application, and 169 net revertants 56 days later (Table 6-4). Thus, when compared to the specific activity of the sample collected immediately following waste application, the specific activity of the CH_2Cl_2 fraction collected 263 days following the first waste application was reduced slightly in the absence of metabolic activation, and relatively unchanged with activation (Figures 6-16 and 6-17).

For the CH_3OH fraction extracted from the Weswood soil which received two sludge applications, the mutagenic potential without activation was

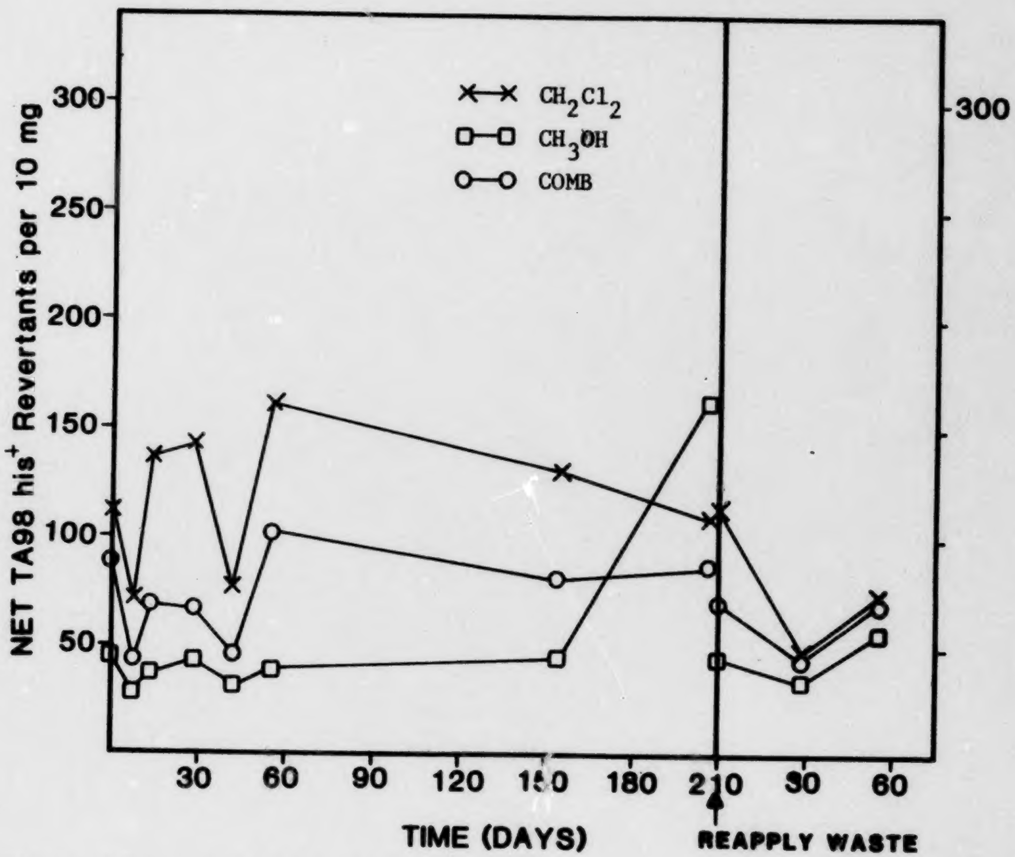

Figure 6-16. Bacterial mutagenicity, as measured without metabolic activation of methylene chloride (CH_2Cl_2), methanol (CH_3OH), and combined fractions (COMB).

essentially unchanged through 156 days following waste application, and increased approximately three-fold in the sample collected 207 days following waste application (Figure 6-16). In the absence of metabolic activation, the CH_3OH fraction extracted from the Weswood soil induced 46 net revertants immediately after waste application, 163 net revertants 207 days after application, and 45 net revertants immediately following the second waste application (Table 6-4). The specific activity of the CH_3OH fraction of the soil extract was not appreciably changed in any of the samples collected during the 56 days following the second waste application (Table 6-4). When compared to the sample collected immediately after waste application, the mutagenic potential with activation of the CH_2Cl_2 extracted from the Weswood soil was increased appreciably through 154 days after waste application and was reduced by approximately 30% in the sample collected 207 days after waste application (Figure 6-17). With metabolic activation, the CH_3OH fraction extracted from the SM3 sludge induced 87 net revertants, while the CH_3OH fraction extracted from the sludge amended soil induced 115, 416, and 269 net revertants immediately following, 154 and 207 days after the first application, respectively; and, 131 net revertants immediately following the second sludge application (Table 6-4). The mutagenic potential of the CH_3OH fraction extracted from the sludge amended Weswood soil 56 days following the second sludge application was 177 net revertants (Table 6-4).

The specific activity without metabolic activation of the combined fraction extracted from the Weswood soil which received two sludge applications remained relatively unchanged during the 263 days following

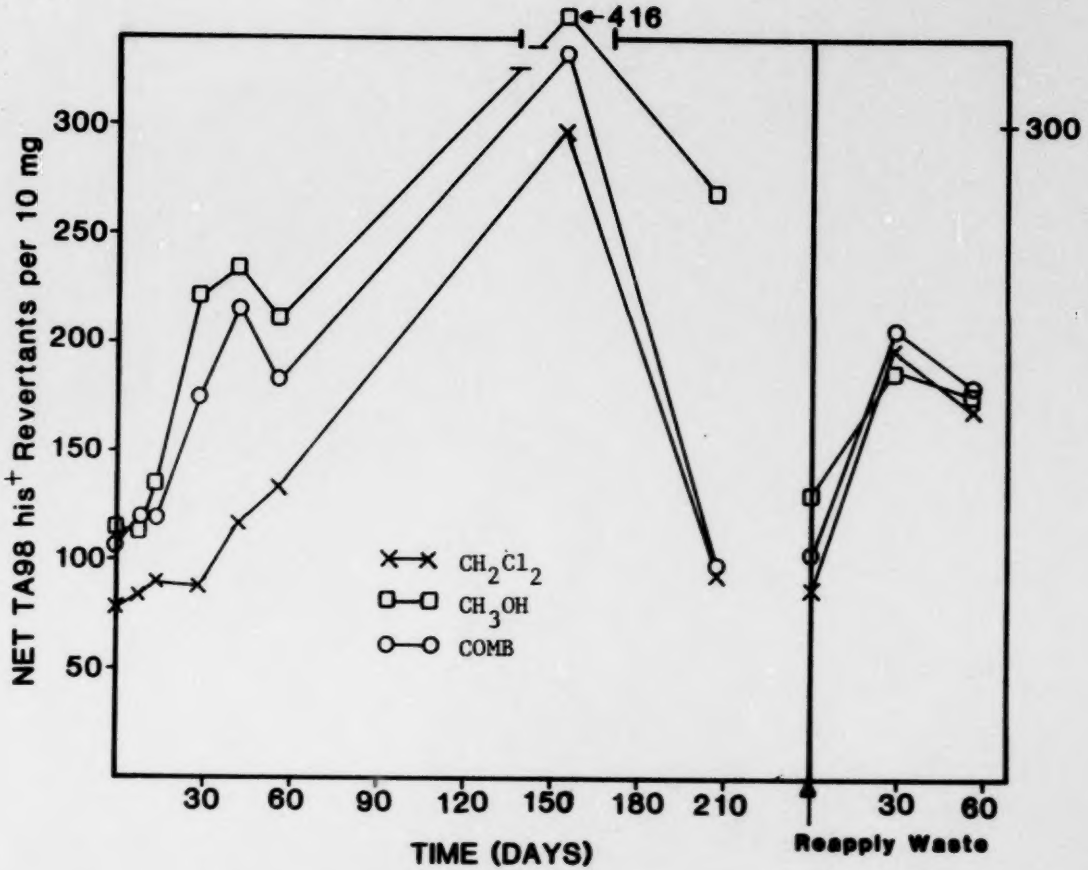

Figure 6-17. Bacterial mutagenicity, as measured with metabolic activation, of methylene chloride (CH_2Cl_2), methanol (CH_3OH), and combined fractions (COMB).

the first sludge application (Figure 6-16). Without metabolic activation, the combined fraction induced 88 net revertants and 87 net revertants immediately following and 207 days after waste application, respectively; while, the combined fraction induced 70 net revertants without activation immediately following the second waste application, and 72 net revertants 56 days after the second waste application (Table 6-4). With activation, the combined fraction induced 106 net revertants, and 96 net revertants .pa immediately following and 207 days after the first waste application; and, 102 and 179 net revertants in the samples collected immediately following and 56 days after the second waste application (Table 6-4). The maximum response observed for a combined fraction from the sludge amended Weswood soil was 333 net revertants without activation induced by the sample collected 154 days after the first waste application (Table 6-4). In general, the specific activity of the combined fraction from the Weswood soil followed the same trend as the CH_2Cl_2 fraction without activation, and followed the same trend as the CH_3OH fraction with activation (Figures 6-16 and 6-17).

The direct-acting mutagenicity of the CH_2Cl_2 fraction extracted from the Weswood soil which received one sludge application of 100 Mg/ha (100 Mg/ha) displayed a continous decrease through 175 days after sludge application (Figure 6-18). Without activation, the specific activity of the CH_2Cl_2 fraction extracted from the Weswood soil decreased initially from 184 net revertants immediately following sludge application to 105 net revertants 116 days later (Table 6-4). The specific activity without metabolic activation was further decreased to 53 net revertants 175 days following sludge application (Table 6-4). The indirect-acting mutagenicity of the CH_2Cl_2 fraction extracted from the Weswood soil which received one sludge application of 100 Mg/ha was increased approximately three-fold from immediately after waste application to 116 days following waste application (Figure 6-18). With metabolic activation, the CH_2Cl_2 fraction extracted from the Weswood soil induced 60 net revertants immediately following waste application, 159 net revertants 56 days after waste application, and 100 net revertants 175 days following waste application (Table 6-4).

The specific activity of the CH_3OH fraction extracted from the Weswood soil which received 100 Mg/ha of sludge in May was reduced slightly in the days immediately following waste application and reached a maximum in the sample collected 56 days after waste application (Figure 6-18). The CH_3OH fraction extracted from the sample collected immediately following sludge application induced 76 net revertants, while the sample collected 56 days after waste application induced 202 net revertants without metabolic activation (Table 6-4). This response was relatively unchanged during the following 60 days of the study as the CH_3OH fraction collected 116 days after sludge application induced 183 net revertants without activation (Table 6-4). However, the CH_3OH fraction extracted from the sludge amended soil 175 days after application induced 42 net revertants without activation (Table 6-4). With metabolic activation, the CH_3OH fraction extracted from the Weswood soil induced 53 net revertants immediately following application, 194 net revertants in the sample collected 116 days following waste application and 64 net revertants in the sample collected 175 days after waste application (Table 6-4). Thus, with and without

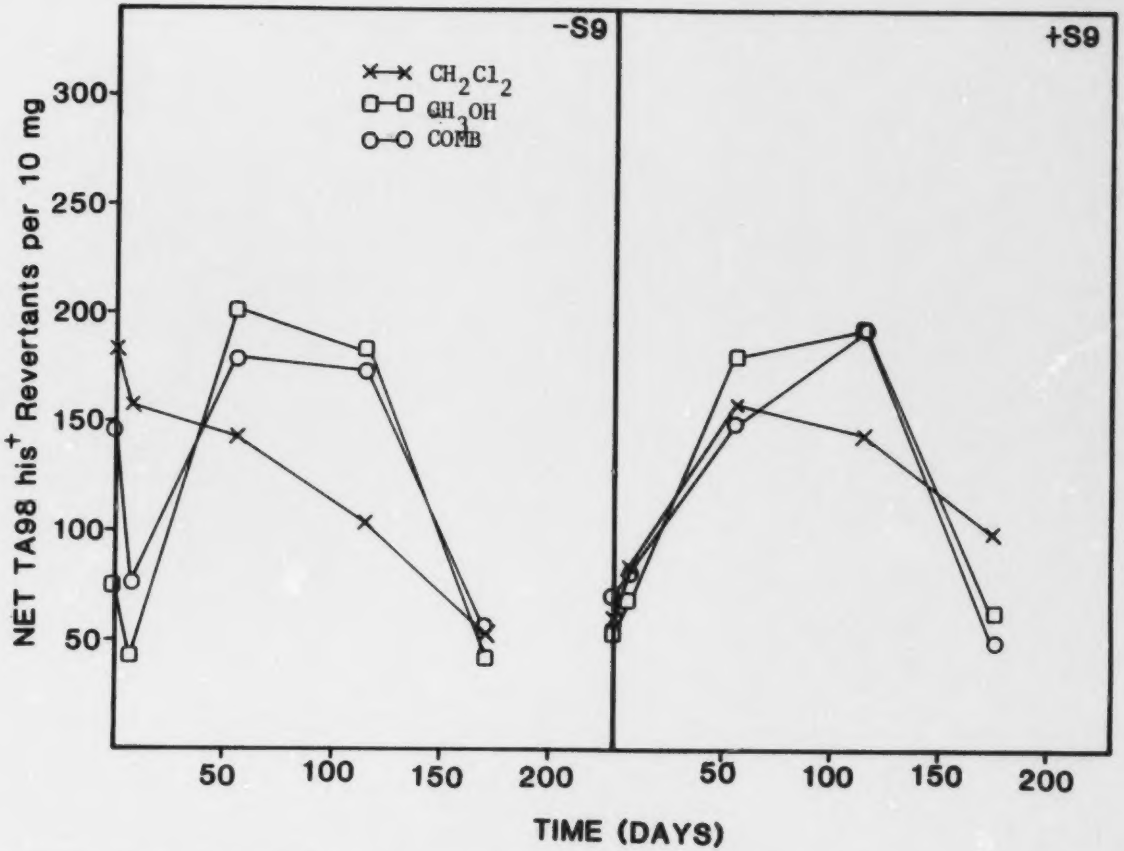

Figure 6-18. Bacterial mutagenicity, as measured with and without metabolic activation of methylene chloride (CH_2Cl_2), methanol (CH_3OH) and combined fractions (COMB) extracted from Weswood loam lysimeters which received one 100 Mg/ha sludge application.

metabolic activation, the specific activity of the CH_3OH fraction extracted from the Weswood soil which received 100 Mg/ha of sludge was increased approximately two-fold during the first four months following waste application and was reduced in the sample collected 175 days after application to a level approximately equal to that observed in the sample collected immediately after application (Figure 6-18).

The direct and indirect activity of the combined fraction extracted from the Weswood soil amended with 100 Mg/ha of sludge increased appreciably in the samples collected 116 days after waste application (Figure 6-18). The specific activity without activation of the combined fraction extracted from the sludge amended Weswood soil which received 100 Mg/ha of sludge in May was 147 net revertants immediately following sludge application and 174 net revertants 116 days after sludge application (Table 6-4). The maximum response induced by a combined fraction without activation was 180 net revertants induced by the sample collected 56 days after waste application (Table 6-4). With metabolic activation, the specific activity of the sludge amended Weswood soil was 70 net revertants immediately following application, 194 net revertants 116 days following application and 49 net revertants 175 days following waste application (Table 6-4). The specific activity of the combined fraction extracted from the sludge amended Weswood soil followed the same general trend as the CH_3OH fraction with and without metabolic activation (Figure 6-18). Thus, although the response observed in the combined fraction extracted from the sample collected 116 days after waste application was approximately twice the response induced by the sample collected immediately following waste application, the response induced by the sample collected 175 days after waste application was only slightly greater than twice background.

The direct and indirect activity of the CH_2Cl_2 fraction extracted from the Weswood soil which received 5.5 kg of sludge (50 Mg/ha) was increased in the sample collected 56 days after waste application (Figure 6-19). At the lowest application rate (50 Mg/ha), the specific activity without activation of the CH_2Cl_2 fraction extracted from the Weswood soil was 167 net revertants immediately following waste application, 186 net revertants 56 days after waste application, and 132 net revertants 175 days following waste application (Table 6-4). In the presence of metabolic activation, the specific activity of the CH_2Cl_2 fraction was 75 net revertants immediately following waste application, and 174 and 172 net revertants for the samples collected 56 and 175 days later, respectively (Table 6-4).

The CH_2Cl_2 extracted from the Weswood soil which received 50 Mg/ha induced 65 net revertants without activation immediately following waste application; while the CH_3OH fractions extracted from samples collected 56 days after waste application induced 222 net revertants, (Table 6-4). The addition of metabolic activation to the assay system reduced the response observed in the sample collected immediately after sludge application to 47 net revertants, while the sample collected 56 days after application induced 169 net revertants and the sample collected 175 days after waste application induced 46 net revertants (Table 6-4).

The specific activity without activation of the combined fraction extracted from the sludge amended Weswood soil was 111 net revertants immediately following sludge application, 200 net revertants 56 days

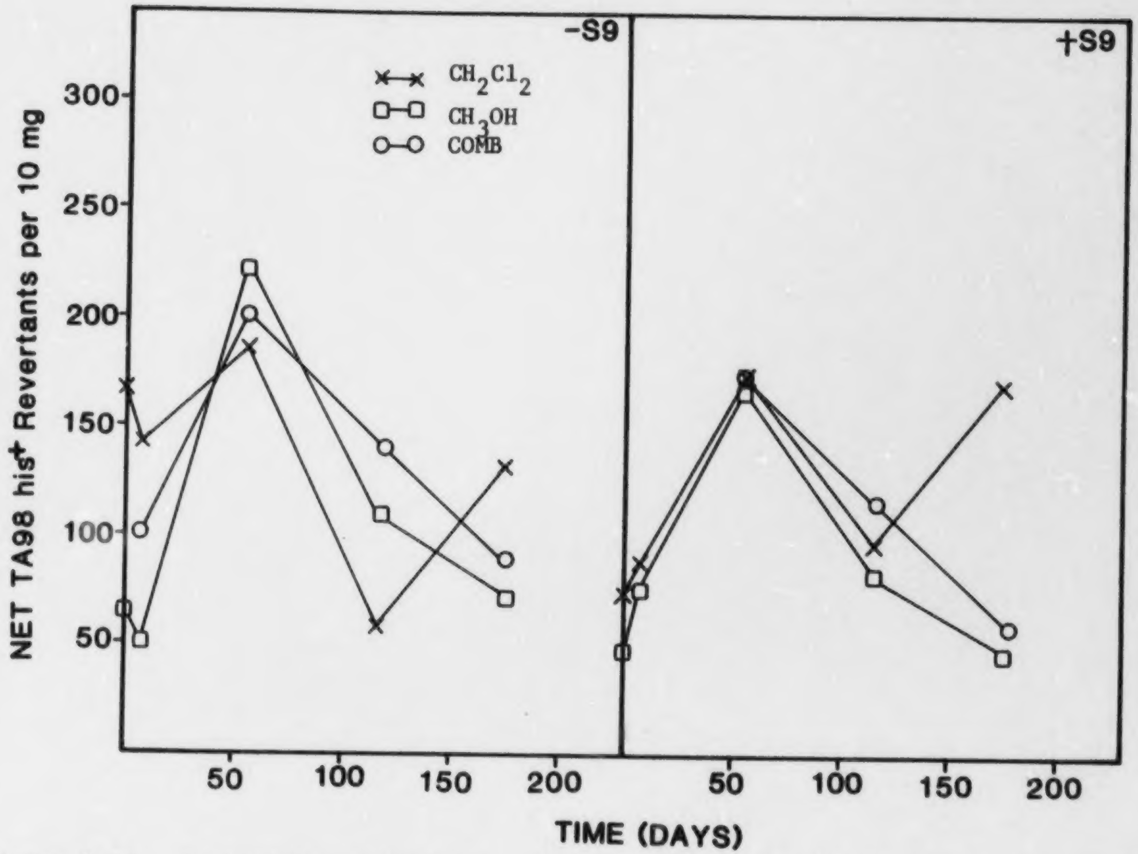

Figure 6-19. Bacterial mutagenicity, as measured with and without metabolic activation of methylene chloride (CH_2Cl_2), methanol (CH_3OH), and combined fractions (COMB) extracted from Weswood loam lysimeters which received one sludge application of 50 Mg/ha.

following waste application, and 88 net revertants 175 days following waste application (Table 6-4). With metabolic activation, the specific activity of the combined fraction extracted from the sludge amended Weswood soil was 60, 171, 116 and 59 net revertants immediately after and 56 116 and 175 days following waste application, respectively (Table 6-4).

The weighted activity of the solvent extractable organics recovered from the sludge amended soil will provide a quantitative indication of the mutagenic potential of the residual organics in the sludge amended soil. The weighted activity was calculated by multiplying the specific activity (revertants per 10 mg from Table 6-4 divided by ten) times the solvent extractable organics (mg/g from Table 6-3) and yields a value equal to the number of revertants which would be induced by one gram of the original material. This value is important for determining the half-life of sludge bound mutagens and accounts for both losses in organic content as well as alterations to the mutagenic potential due to degradation. The results presented in Table 6-5 indicate that the municipal sewage sludges employed for this study contained a persistent mutagenic residue. For the Padina sand which received two waste applications totalling 16.5 kg (150 Mg/ha), the weighted activity without activation of the CH_2Cl_2 was reduced from 154 revertants per gram immediately following waste application to 93 revertants per gram on day 207 (Table 6-5 and Figure 6-20). However, with metabolic activation, the activity was increased from 81 revertants per gram immediately following waste application to 120 revertants per gram 207 days later (Table 6-5 and Figure 6-21). For the CH_3OH and combined fractions, the weighted activity without activation was reduced appreciably

TABLE 6-5. WEIGHTED ACTIVITY OF SOLVENT EXTRACTABLE ORGANICS RECOVERED FROM MUNICIPAL SEWAGE SLUDGE AMENDED LYSIMETERS †

		Days After First Application																					
		0		14		28		56		116		154		175		207		210		236		263	
																		Days After Second Application					
																		0		28		56	
Treatment	Fraction	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	S9	-S9	+S9
Padina 150 Mg/ha (Dec/Aug)	CH_2Cl_2	154††	81	146	118	246	140	192	173	NS	NS	115	203	NS	NS	93	120	124	213	NS	NS	67	205
	CH_3OH	105	147	60	195	87	371	47	329	NS	NS	46	384	NS	NS	19	190	36	177	87	489	113	636
	COMB	310	371	216	501	306	581	240	588	NS	NS	90	530	NS	NS	27	321	230	543	187	707	205	775
Padina 100 Mg/ha (May)	CH_2Cl_2	251	105	NS	NS	NS	NS	81	135	34	89	NS	NS	36	77	NS	NS	NS	NS	NS	NS	NS	NS
	CH_3OH	133	75	NS	NS	NS	NS	34	70	25	70	NS	NS	26	70	NS	NS	NS	NS	NS	NS	NS	NS
	COMB	403	185	NS	NS	NS	NS	144	218	60	177	NS	NS	51	128	NS	NS	NS	NS	NS	NS	NS	NS
Padina 50 Mg/ha (May)	CH_2Cl_2	110	71	NS	NS	NS	NS	39	42	41	44	NS	NS	17	22	NS	NS	NS	NS	NS	NS	NS	NS
	CH_3OH	43	29	NS	NS	NS	NS	47	46	16	18	NS	NS	15	23	NS	NS	NS	NS	NS	NS	NS	NS
	COMB	178	78	NS	NS	NS	NS	92	95	23	48	NS	NS	35	51	NS	NS	NS	NS	NS	NS	NS	NS
Weswood 150 Mg/ha (Dec/Aug)	CH_2Cl_2	122	87	260	171	215	132	193	160	NS	NS	118	267	NS	NS	55	47	328	252	85	355	141	338
	CH_3OH	170	426	89	324	86	442	48	295	NS	NS	130	333	NS	NS	130	215	81	236	68	376	148	460
	COMB	422	509	297	512	235	613	225	455	NS	NS	138	566	NS	NS	113	125	329	479	167	762	331	823
Weswood 100 mT/ha (May)	CH_2Cl_2	239	78	NS	NS	NS	NS	72	80	53	73	NS	NS	27	50	NS	NS	NS	NS	NS	NS	NS	NS
	CH_3OH	84	58	NS	NS	NS	NS	101	91	73	78	NS	NS	17	26	NS	NS	NS	NS	NS	NS	NS	NS
	COMB	353	168	NS	NS	NS	NS	198	165	157	175	NS	NS	54	49	NS	NS	NS	NS	NS	NS	NS	NS
Weswood 50 mT/ha (May)	CH_2Cl_2	217	98	NS	NS	NS	NS	74	70	35	59	NS	NS	53	69	NS	NS	NS	NS	NS	NS	NS	NS
	CH_3OH	59	42	NS	NS	NS	NS	111	85	43	33	NS	NS	29	9	NS	NS	NS	NS	NS	NS	NS	NS
	COMB	244	132	NS	NS	NS	NS	180	154	139	116	NS	NS	53	35	NS	NS	NS	NS	NS	NS	NS	NS
Padina Control	CH_2Cl_2	13	10	NS	NS	2	2	2	3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CH_3OH	6	4	NS	NS	25	6	8	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	COMB	18	3	NS	NS	20	9	23	22	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Weswood Control	CH_2Cl_2	7	7	NS	NS	4	7	10	10	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	CH_3OH	88	54	NS	NS	26	14	44	36	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	COMB	62	49	NS	NS	41	17	43	34	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

† NS = Not sampled; CH_2Cl_2 = methylene chloride; CH_3OH = methanol; COMB = combined ††Data represents mean of triplicate extractions on three replicate treatments (N=9).

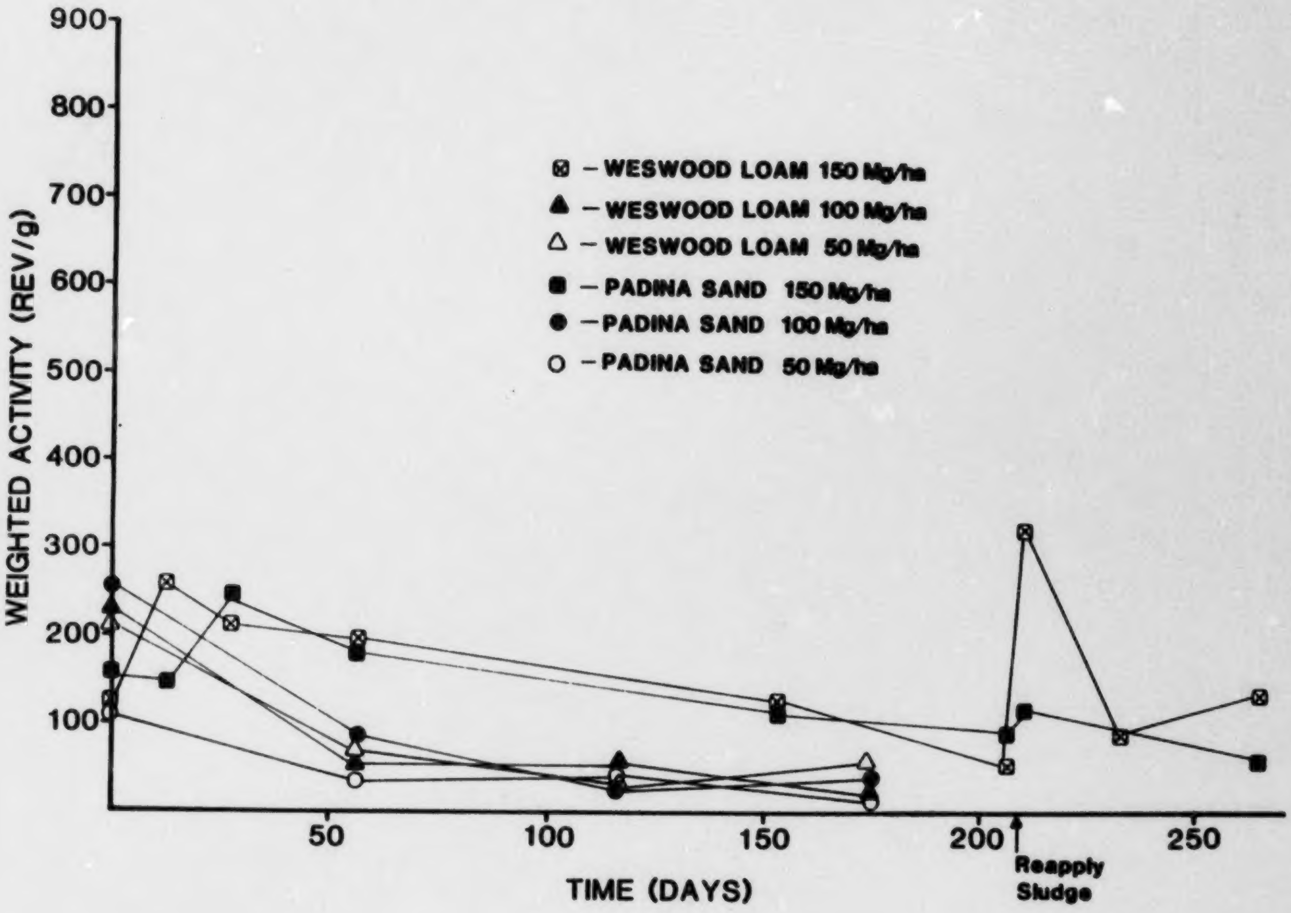

Figure 6-20. Weighted activity without metabolic activation of CH_2Cl_2 fraction extracted from sludge amended Padina or Weswood soil.

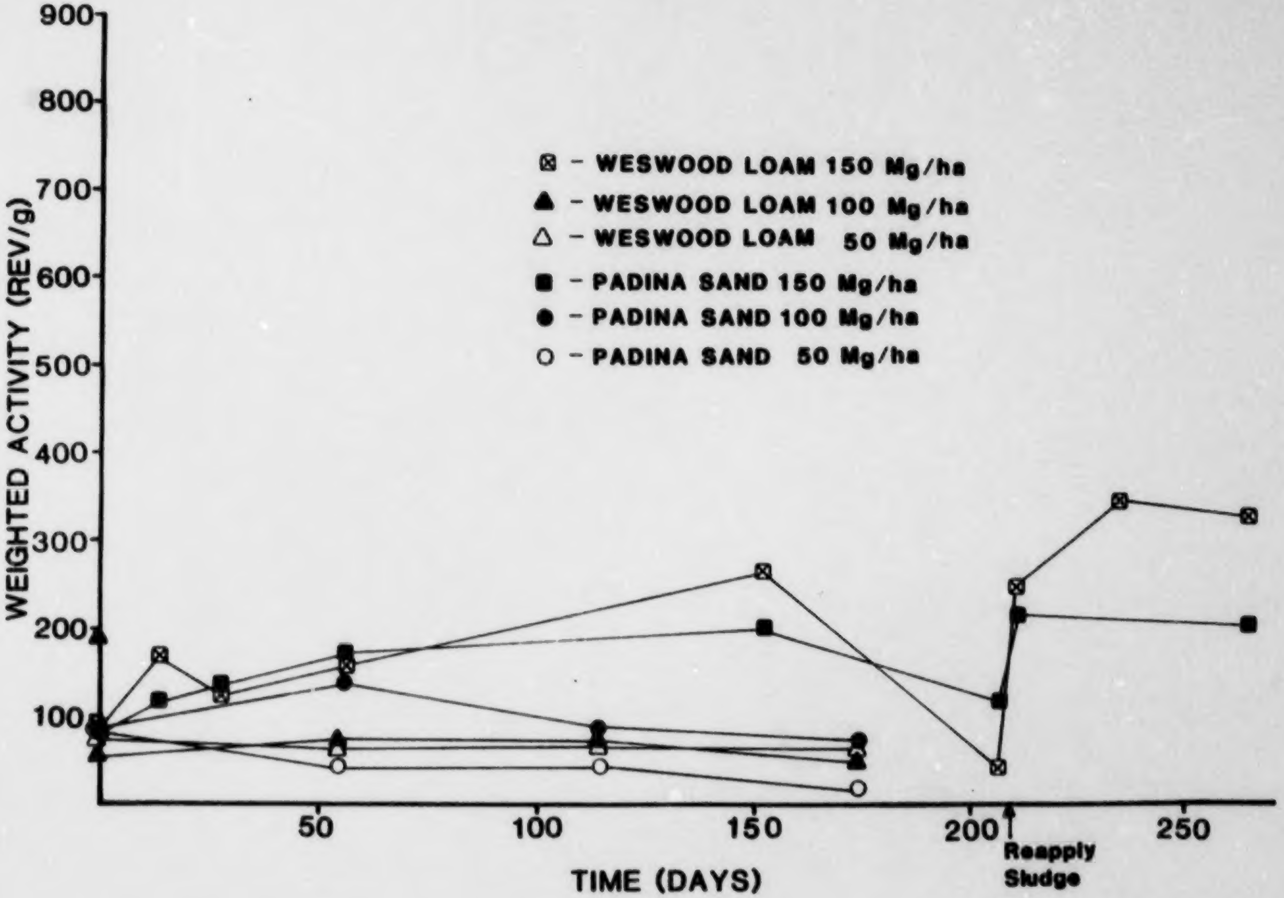

Figure 6-21. Weighted activity with metabolic activation of CH_3OH fraction extracted from sludge amended Padina or Weswood soil.

207 days after waste application, although the weighted activity with activation was essentially unchanged (Table 6-5 and Figures 6-22 through 6-24). Following the second sludge application to the Padina sand, the weighted activity of the CH_2Cl_2 fraction without activation was reduced on day 56 by approximately 50%, while the activity with activation was unchanged. For the CH_3OH fraction, both the direct and indirect activity of the sample collected 56 days after waste application were increased from three to four times the level observed in the sample collected immediately following waste application (Figures 6-22 and 6-23). In the absence of activation, the combined fraction from the Padina sand was essentially unchanged 56 days following waste application, while weighted activity with metabolic activation was increased from 543 revertants per gram immediately following the second waste application, to 775 revertants per gram 56 days following the second waste application (Table 6-5 and Figure 6-25).

The weighted activity of the Padina sand which received one application of 100 Mg/ha sludge in May is provided in Table 6-5, Figures 6-20, 6-22 and 6-24. These data indicate that the weighted activity without activation was appreciably reduced for all three fractions collected 175 days following waste application when compared to the weighted activities induced immediately following activation. However, in the presence of metabolic activation, the weighted activities were 105, 75, and 185 revertants per gram for the CH_2Cl_2, CH_3OH and combined fractions, respectively, collected immediately after waste application, and 77, 70, and 128 revertants per gram for the CH_2CL_2, CH_3OH, and combined fractions collected 175 days after waste application (Table 6-5). The weighted

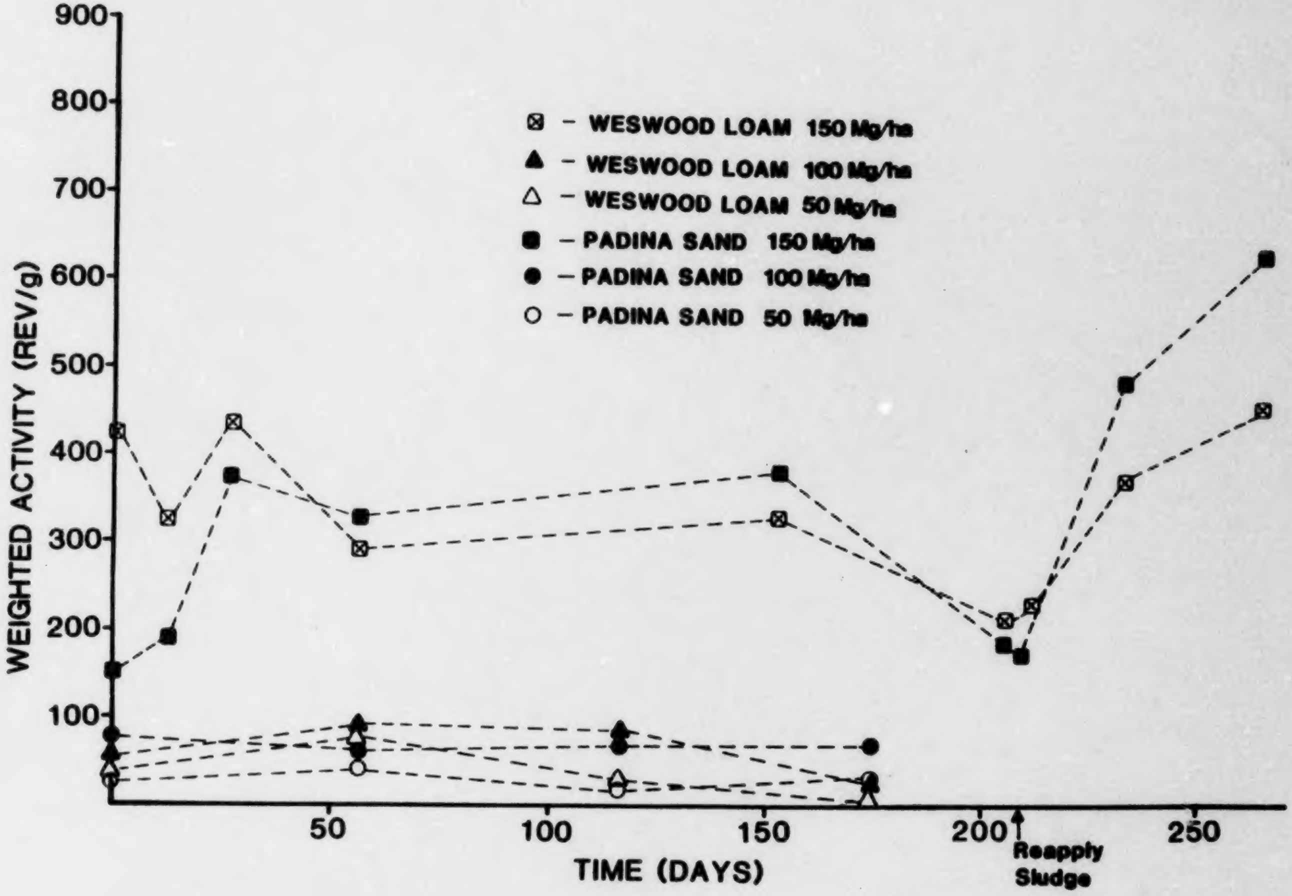

Figure 6-22. Weighted activity without metabolic activation of combined fractions (COMB) extracted from sludge amended Padina or Weswood soil.

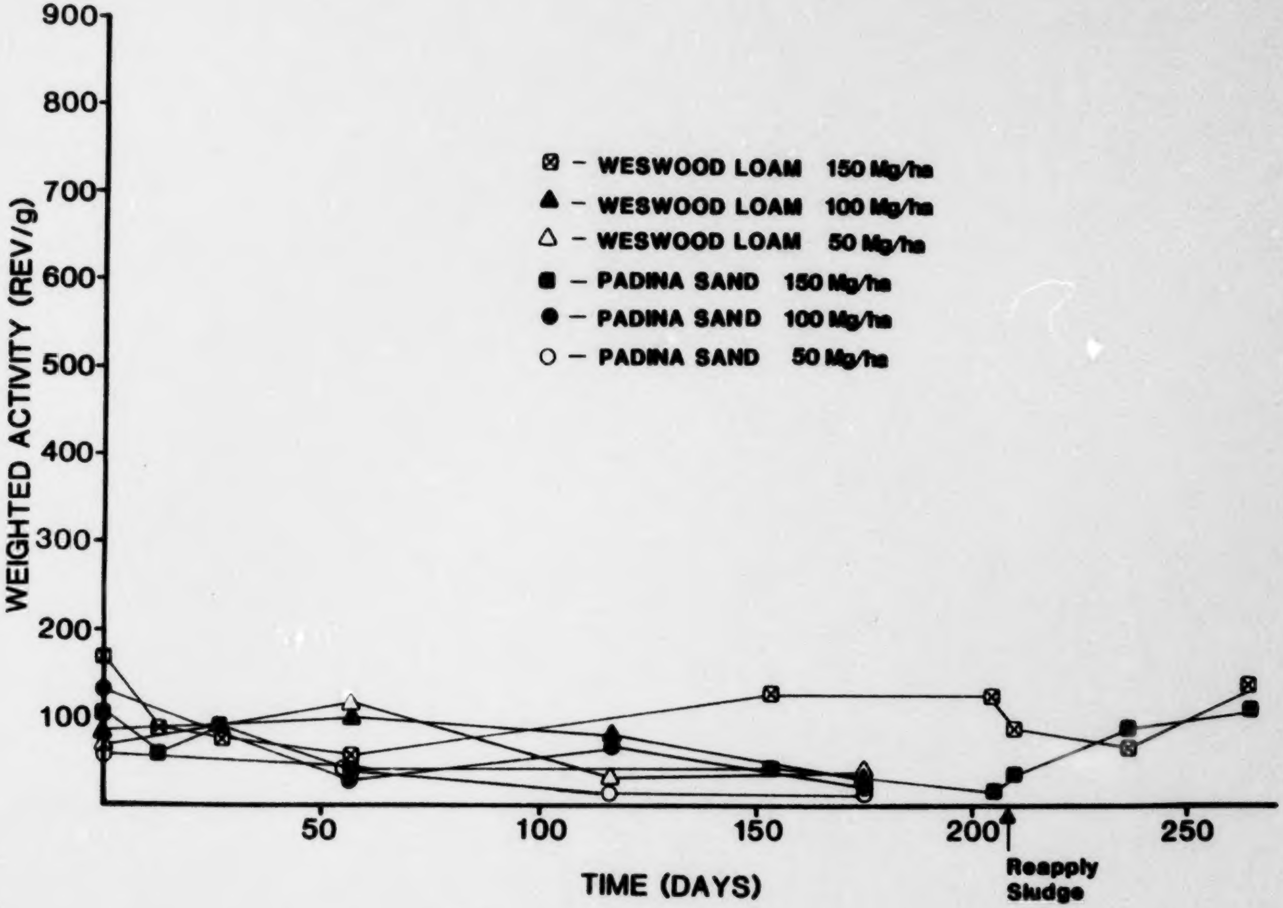

Figure 6-23. Weighted activity with metabolic activation of methylene chloride (CH_2Cl_2) fraction extracted from sludge amended Padina or Weswood soil.

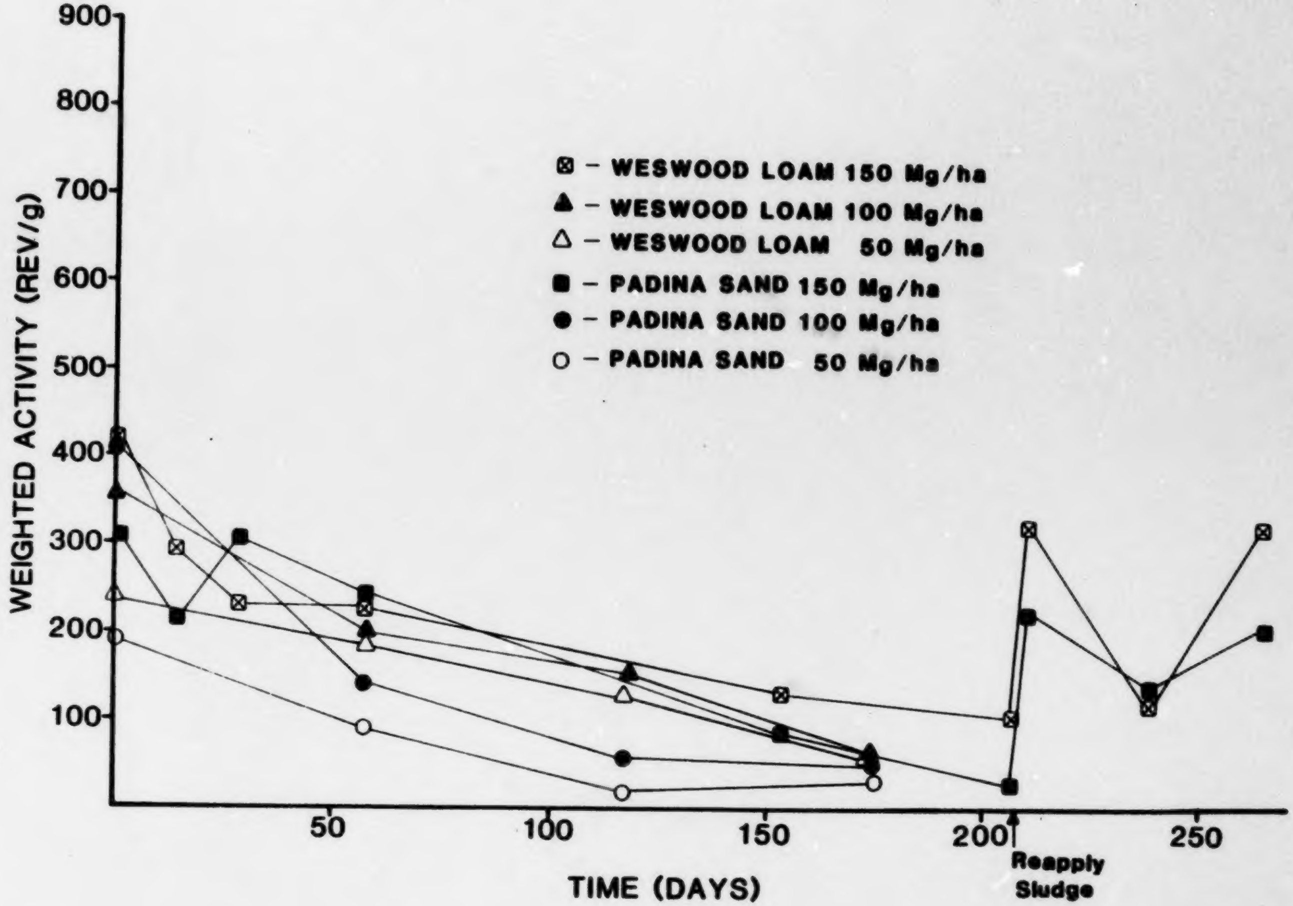

Figure 6-24. Weighted activity without metabolic activation of methanol (CH_3OH) fraction extracted from sludge amended Padina or Weswood soil.

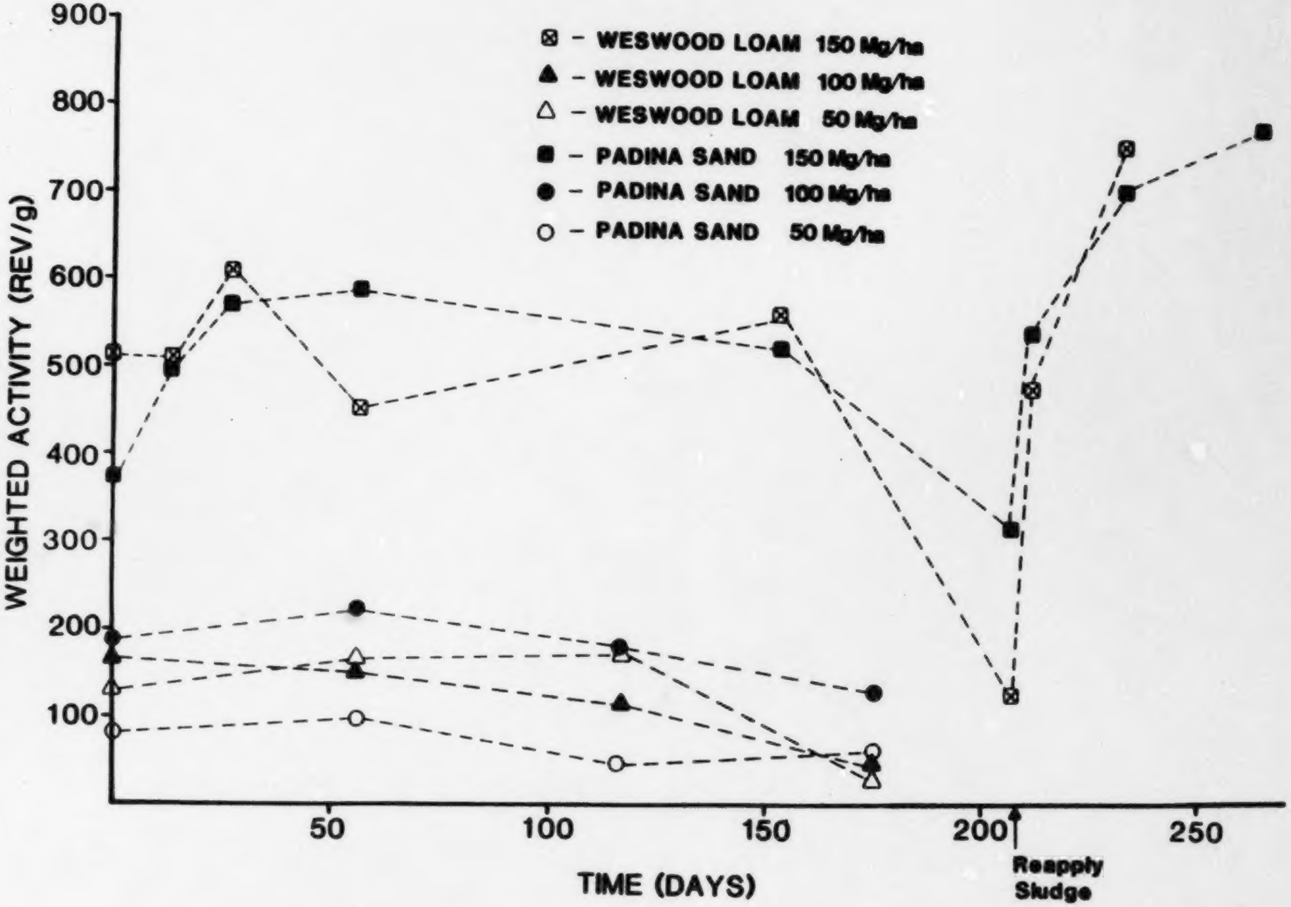

Figure 6-25. Weighted activity with metabolic activation of combined fractions (COMB) extracted from sludge amended Padina or Weswood soil.

activities of the fractions extracted 116 days after application from the Padina sand which received one sludge application of 5.5 kg (50 Mg/ha) were approximately equal to the weighted activities of the comparable fraction from the unamended soil (Table 6-5). Without activation, the weighted activities were 110, 43 and 178 revertants per gram for the CH_2Cl_2, CH_3OH, and combined fraction collected immediately after sludge application (Table 6-5). The weighted activities without activation of the fractions extracted from the soil collected 175 days after sludge application were 17, 15, and 35 revertants per gram for the CH_2Cl_2, CH_3OH, and combined fractions, respectively (Table 6-5). For comparison, the maximum weighted activities without activation observed in the unamended Padina sand were 13, 25, and 23 revertants per gram for the CH_2Cl_2, CH_3OH and combined fractions, respectively (Table 6-5). With activation, the weighted activities of the fractions extracted from the soil collected 175 days following sludge application were also approximately equal to the maximum weighted activities of the unamended Padina soil (Table 6-5 and Figures 6-21, 6-23, and 6-25).

The weighted activities without activation of all three fractions extracted from the Weswood loam which received two sludge applications totalling 16.5kg (150 Mg/ha) was appreciably reduced 207 days following the initial waste application (Table 6-5). For the CH_2Cl_2 fraction without metabolic activation, the weighted activity increased from 122 revertants per gram immediately following waste application to 260 revertants per gram 14 days after application; and, was decreased to 55 revertants per gram 207 days following waste application (Table 6-5). Without metabolic activation, the CH_3OH fraction of the Weswood soil induced 170 revertants per gram immediately following waste application, and 130 revertants per gram 207 days following waste application (Table 6-5). In the presence of metabolic activation, the CH_3OH fraction extracted from the Weswood soil induced 426 revertants per gram immediately following application, and 215 revertants per gram 207 days following waste application (Table 6-5).

The combined fraction of the Weswood soil which received two sludge applications induced the maximum weighted activity of all fractions collected immediately following waste application (Figures 6-24 and 6-25. The weighted activity of the combined fraction was 422 revertants per gram without activation, and 509 revertants per gram with activation (Table 6-5). Without metabolic activation, the weighted activtiy of the combined fraction decreased to 113 revertants per gram 207 days after application, while the weighted activity with activation reached a maximum of 613 revertants per gram 28 days after application, and decreased to 125 revertants per gram in the sample collected 207 days following waste application (Table 6-5). Following the second sludge application to the Weswood soil, the weighted activity increased initially to 329 revertants per gram without activation and 479 revertants per gram with activation (Table 6-5). The weighted activity of the combined fraction collected 56 days after the second sludge application was essentiallly unchanged without activation, and increased appreciably to 823 revertants per gram with activation. These data indicate that while a slight reduction in the weighted activity was observed 207 days following the first waste application, although 56 days following the second sludge application the weighted activity with activation was increased in two of three fractions (Figure 6-25).

For the Weswood loam which received one application of 100 Mg/ha of sludge in May, the results indicate that for all three fractions the weighted activity was reduced in the sample collected 175 days following sludge application. The weighted activity without metabolic activation of the CH_2Cl_2 fraction was reduced from 239 revertants per gram immediately following waste application to 53 revertants per gram 116 days after waste application and 27 revertants per gram 175 days after waste application (Table 6-5). While the weighted activity with activation was 78 immediately following waste application, 73 revertants per gram in the sample collected 116 days following waste application, and only 27 revertants per gram in the sample collected 175 days after waste application (Table 6-5). For the combined fraction, the weighted activity without activation was reduced from 353 revertants per gram immediately following waste application to 54 revertants per gram on day 175 (Table 6-5). While with metabolic activation the weighted activity of the combined fraction was 168 revertants per gram immediatelty following waste application, 175 revertants per gram 116 days following waste application and 49 revertants per gram 175 days following waste application (Table 6-5).

Similar results were observed for the Weswood soil which received one sludge application of 50 Mg/ha (5.5 kg). Without metabolic activation, the weighted activities of the CH_2Cl_2 fraction were 217, 74, 35, and 53 revertants per gram immediately after, 56, 116, and 175 days following waste application (Table 6-5). In the presence of metabolic activation, the weighted activities of the CH_2Cl_2 fractions were 98, 70, 59, and 69 revertants per gram immediately after, 56, 116, and 175 days following waste application (Table 6-5). For the CH_3OH fraction, the weighted activity was increased approximately two-fold with and without activation for the sample collected 56 days after application, although the sample collected 175 days after waste application induced 29 revertants per gram without activation, and 9 revertants per gram with activation (Table 6-5 and Figures 6-22 and 6-23). The combined fraction induced a weighted activity of 244 and 132 revertants per gram without and with metabolic activation immediately after waste application (Table 6-5). The weighted activity of the combined fraction .pa was reduced slightly for the sample collected 116 days after waste application, while the sample collected 175 days after waste application induced a weighted activity of 53 revertants per gram without activation and 35 revertants per gram with activation (Table 6-5).

These data indicate that a minimum of eight weeks were required for the weighted activity of the soil to reach a maximum, and that the residual mutagens in the soil were persistent, and could still be detected at appreciable levels as much as 207 days following waste application. Three of the treatments, the Padina sand which received one application of 50 Mg/ha of sludge and the Weswood soil which received 50 Mg/ha or 100 Mg/ha of sludge, appeared to return to a weighted activity which was approximately equal to the weighted activity observed in the background soil samples.

MOBILITY

In general, land treatment of waste is a race between degradation and immobilization. Environmental risk from land application of sewage is minimized when chemical(s) are transformed, degraded, or removed from the soil system at a rate which is greater than the rate at which the chemical(s) are dissolved into the soil-pore water and thus available for leaching. The quantity of organics extracted from each of the leachate samples is provided in Table 6-6. These results indicate that for the majority of lysimeters, the quantity of organics present in the leachate water collected from the waste amended lysimeters was appreciably greater than the quantity of organics present in the leachate water collected from the control lysimeters.

The quantity of solvent extractable organics recovered from the leachate water from the Padina sand lysimeters which received two waste applications (total applied 150 Mg/ha) was increased to three to four times the background level in the first half of a pore volume; and, was further increased to three to four times the previous level in the 0.5 to 1.0 pore volume sample (Table 6-6). The mean solvent extractable organics level in the 0 to 0.5 pore volume samples was 2.29 mg/L (Table 6-6). The same general trend was observed in the leachate from the Weswood loam lysimeters which received two waste applications. Although the quantity of solvent extractable organics recovered from Replication 1 decreased, all leachate samples collected from the Weswood loam lysimeters with two waste applications were found to contain appreciably greater quantities of residual organics than the composite background sample (Table 6-6). In

TABLE 6-6. ACETONE EXTRACTABLE ORGANICS RECOVERED FROM LYSIMETER LEACHATE SAMPLES CONCENTRATED ON XAD COLUMNS

Lysimeter	Treatment	0-0.5 P.V.[†]	0.5-1.0 P.V.	1.0-1.5 P.V.
Control	Padina sand (50 L)	0.66		
Control	Weswood loam (50 L)	1.41		
Rep. 1	Padina sand 150 Mg/ha	1.77	7.80	-
Rep. 2		2.35	9.89	-
Rep. 3		2.76	6.24	-
Mean		2.29	7.98	-
Rep. 1	Padina sand 100 Mg/ha	-	-	-
Rep. 2		7.20	-	-
Rep. 3		3.98	-	-
Mean		5.59	-	-
Rep. 1	Padina sand 50 Mg/ha	1.15	-	-
Rep. 2		2.48	5.92	-
Rep. 3		2.03	3.46	-
Mean		1.89	4.69	-
Rep. 1	Weswood loam 150 Mg/ha	5.63	2.63	-
Rep. 2		3.19	5.38	5.50
Rep. 3		2.66	4.29	-
Mean		3.83	4.10	-
Rep. 1	Weswood loam 50 Mg/ha	0.93	1.23	-
Rep. 2		0.84	1.37	-
Rep. 3		0.70	2.13	3.08
Mean		0.82	1.58	-

[†] PV = Pore Volume; one half pore volume is equal to 47.5 liters.

addition, the residue weight for the one lysimeter from which three samples were collected (Replication 2) increased from 3.19 mg/L to 5.38 mg/L and 5.50 mg/L for pore volumes 0 to 0.5, 0.5 to 1.0 and 1.0 to 1.5, respectively (Table 6-6).

For the Padina sand which received one application at 50 Mg/ha, the weight of residual organics in the leachate water was consistently greater than was recovered from the control sample and slightly less than was recovered from the Padina sand which received two sludge applications. The leachate water from the Padina sand which received one application at 100 Mg/ha was found to contain a greater quantity of residual organics than the leachate from the Padina lysimeters which received one application at 50 Mg/ha. The mean quantity of solvent extractable organics recovered from the low application Padina sand lysimeters was 1.89 mg/L and 4.69 mg/L for the 0 to 0.5 and 0.5 to 1.0 pore volumes, respectively (Table 6-6). The quantity of organics recovered from the 0 to 0.5 and 0.5 to 1.0 pore volume leachate water samples from the Weswood loam which received one application of 50 Mg/ha was not appreciably different from the control sample. However, the 1.0 to 1.5 pore volume leachate water sample from Replication 3 did contain 3.08 mg/L solvent extractable organics, or approximately twice the level of organics observed in the composite background sample (Table 6-6). Thus, it appears that the addition of sewage sludge to the soil lysimeters resulted in greater quantities of residual organics being recovered in the leachate water.

The presence of increased quantities of organics in the leachate water also appears to have been reflected in the specific activity of the leachate samples. Only one of the 0 to 0.5 pore volume leachate samples had a specific activity that would be indicative of a mutagenic response. The first leachate sample tested from Replication 1 of the (Weswood loam, two applications) induced 50 net revertants without S9 and 64 net revertants with S9 at a dose of 10 mg per plate (Table 6-7 and Figure 6-26). The Br breakthrough curves (Figures 6-1 through 6-8) indicate that breakthrough occurred earlier in the Weswood soils than in the Padina sand, and that Replication 1 showed a Br breakthrough at approximately 0.4 pore volumes. Borderline mutagenic responses were also observed for the 0 to 0.5 pore volume samples from all three Westwood loam lysimeters, treated with one 50 Mg/ha application) (Table 6-7).

Three of eleven 0.5 to 1.0 pore volume leachate samples induced a positive response in the bioassay. For all three of these samples, a greater response was observed in the absence of metabolic activation than with metabolic activation. This is the same general pattern observed for the CH_2Cl_2 and combined fraction of the SM sludges applied. Both Replication 2 of the Padina sand treated with 150 Mg/ha application and Replication 2 of the Weswood loam induced 207 net revertants without activation, and 135 net revertants with metabolic activation (Table 6-7 and Figures 6-27 and 6-28). Each of these lysimeters had received two applications for a total of 150 Mg/ha of sludge. In addition, the 0.5 to a 1.0 pore volume leachate water from the Padina sand which recieved one application of 50 Mg/ha induced 129 net revertants without activation, and 57 net revertants with metabolic activation (Table 6-7 and Figure 6-29). A weak positive response was also observed in the 0.5 to 1.0 pore volume sample from Replication 1 of the Weswood soil treated with 150 Mg/ha and Replications 2 and 3 of the Weswood soil treated with 50 Mg/ha. Only two lysimeters yielded sufficient leachate water to extract three samples. The 1.0 to 1.5 pore volume sample from Replication 3 of the Weswood loam

TABLE 6-7. SPECIFIC MUTAGENIC ACTIVITY OF ACETONE EXTRACTABLE ORGANICS FROM LEACHATE SAMPLES CONCENTRATED ON XAD COLUMNS

	Specific Activity					
	0-0.5 PV		0.5-1.0 PV		1.0-1.5 PV	
Lysimeter	-S9	+S9	-S9	+S9	-S9	+S9
Background						
Padina	BD	3				
Weswood	14	14				
Rep. 1 (Padina	BD	3	12	BD	-	-
Rep. 2 150 Mg/ha)	13	6	131	43	-	-
Rep. 3	5	BD	9	40	-	-
Rep. 1 (Padina	-	-	-	-	-	-
Rep. 2 100 Mg/ha)	2	30	-	-	-	-
Rep. 3	BD	BD	-	-	-	-
Rep. 1 (Padina	8	BD	-	-	-	-
Rep. 2 50 Mg/ha)	18	12	129	57	-	-
Rep. 3	2	7	15	28	-	-
Rep. 1 (Weswood	50	64	50	42	-	-
Rep. 2 150 Mg/ha)	9	BD	207	135	64	27
Rep. 3	13	7	3	39	-	-
Rep. 1 (Weswood	23	33	25	22	-	-
Rep. 2 50 Mg/ha)	31	35	43	34	-	-
Rep. 3	21	14	36	17	97	50

Specific activity equals net TA98 revertants per 10 mg.
PV = Pore Volume; one half pore volume is equal to 47.5 liters.
Control values: DMSO (-S9) = 28; DMSO (+S9) = 36;
2-nitrofluorene (25µg/plate) = 1836; 2-aminoanthracene (+S9-10µg/plate) = 1397 total revertants. DB = Below detection.

treated with one 50 Mg/ha application induced 97 net revertants without, and 50 net revertants with metabolic activation (Table 6-4 and Figure 6-30); while the 1.0 to 1.5 pore volume sample from Replication 2 of the

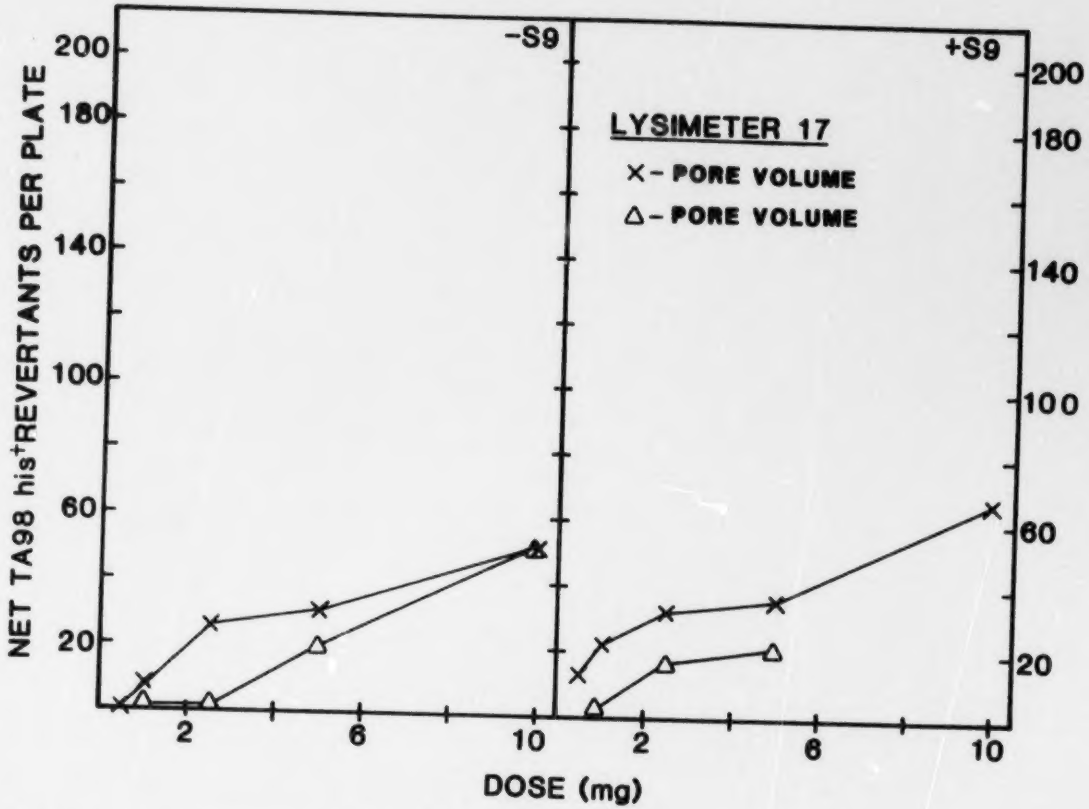

Figure 6-26. Bacterial mutagenicity of acetone, extractable organics recovered from Weswood loam (Lysimeter 17) leachate samples.

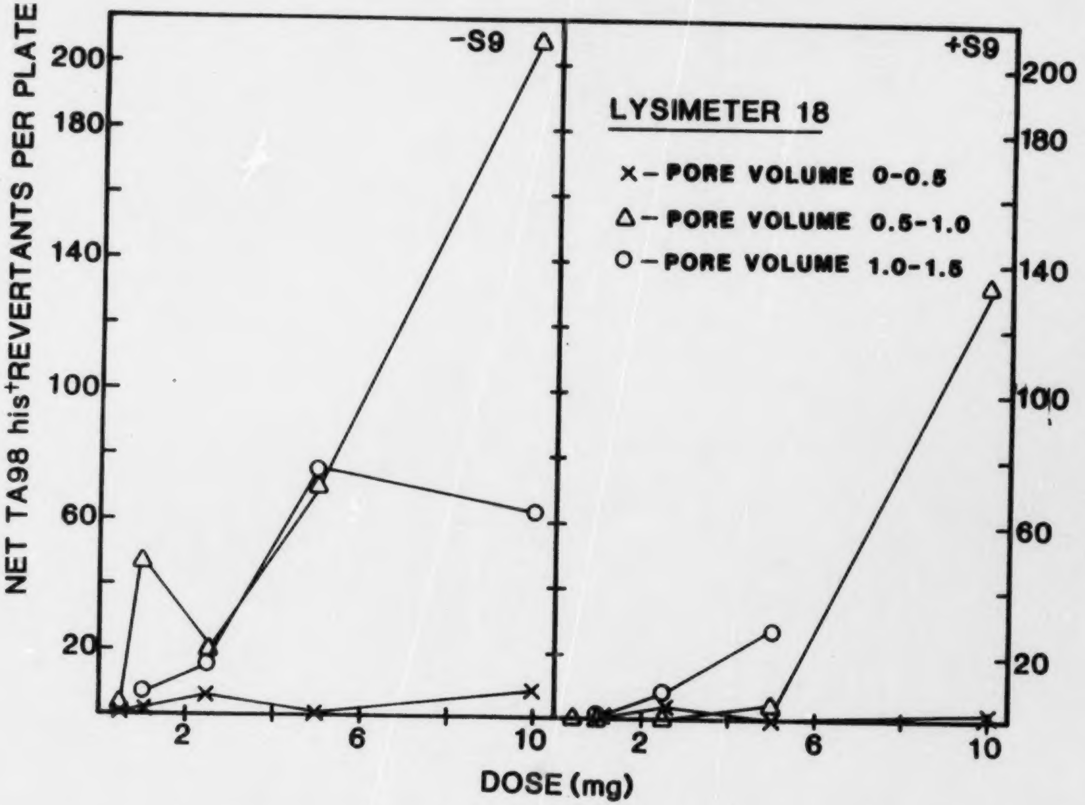

Figure 6-27. Bacterial mutagenciity of acetone extractable organics recovered from Weswood loam (Lysimeter 18) leachate samples.

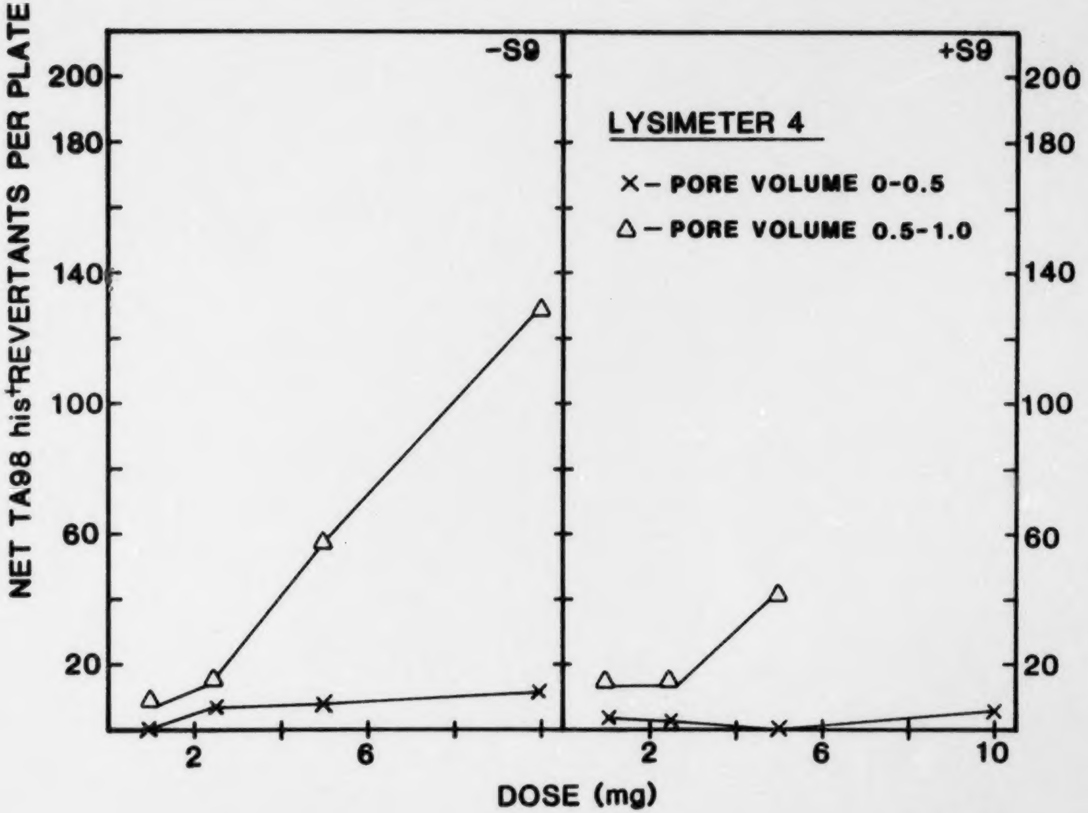

Figure 6-28. Bacterial mutagenicity of acetone extractable organics recovered from Padina sand (Lysimeter 4) leachate sample.

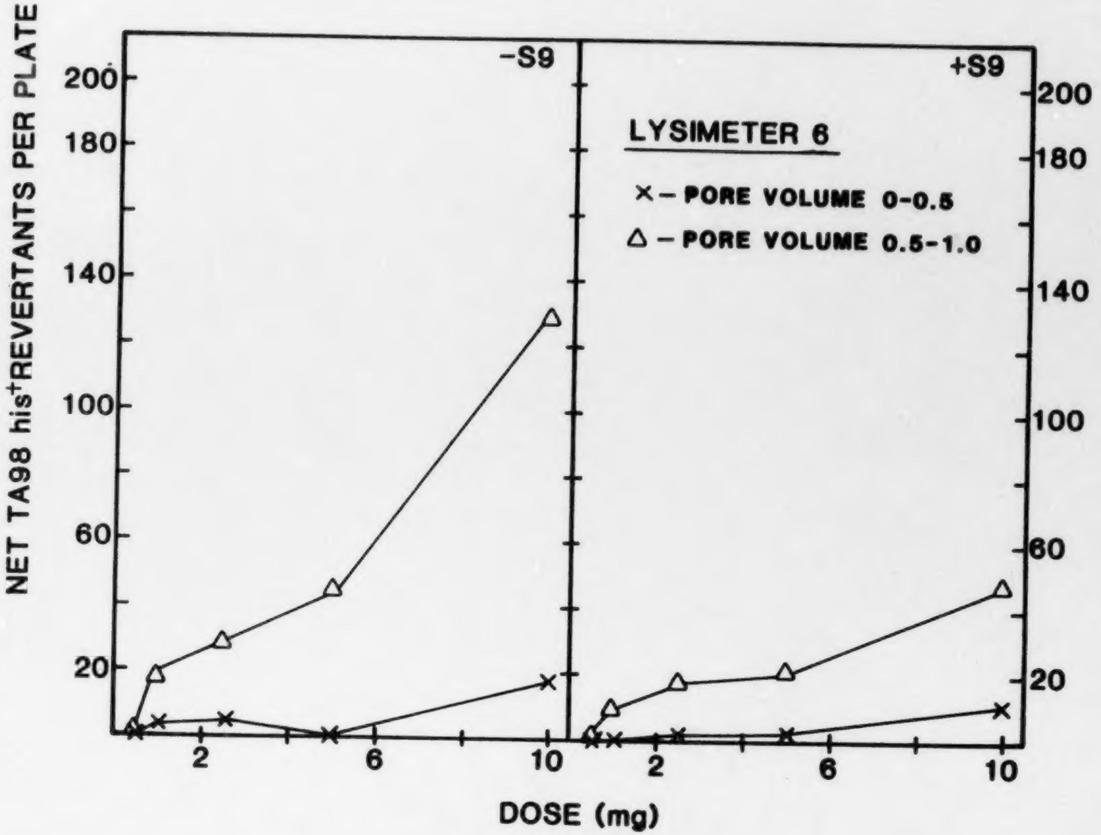

Figure 6-29. Bacterial mutagenicity of acetone extractable organics recovered Padina sand (Lysimeter 6) leachate sample.

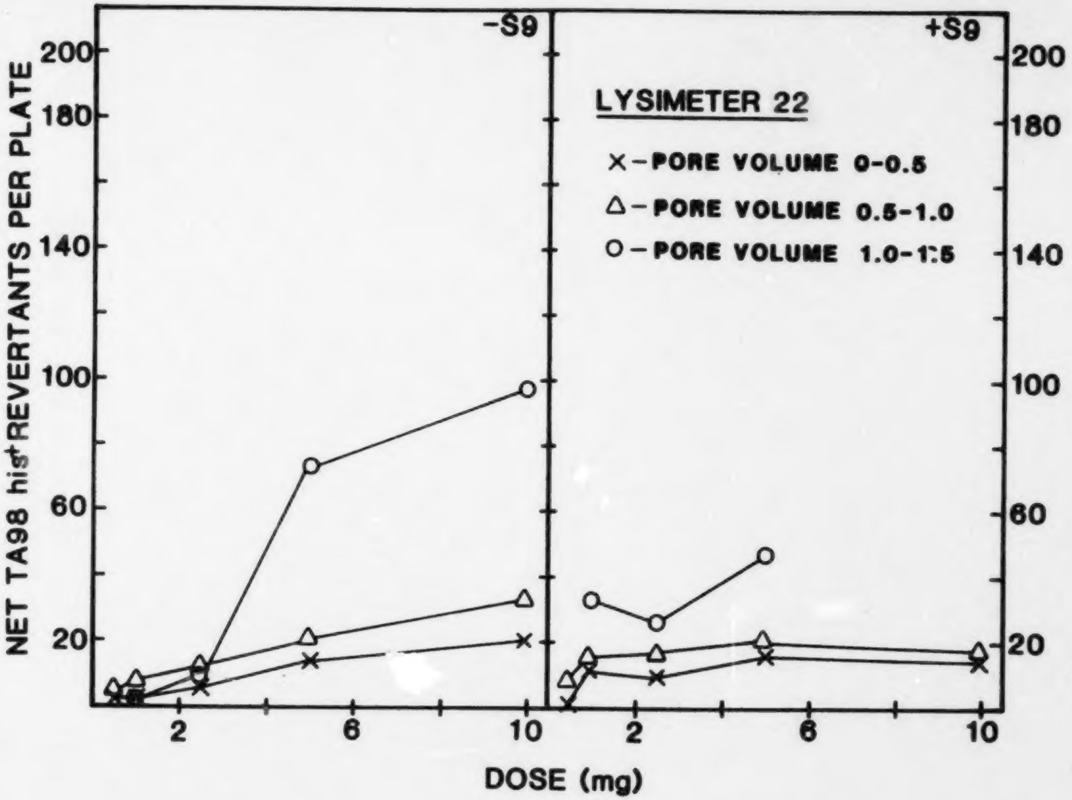

Figure 6-30. Bacterial mutagenicity of acetone extractable organics recovered from Weswood loam (Lysimeter 22) leachate samples.

Weswood loam which received 150 Mg/ha of sludge induced 64 and 27 net revertants without and with metabolic activation. These results indicate that leaching of organic mutagens from sludge amended soils is a highly variable phenomenon. For most treatments, there was little or no correlation between replicates. The rate of leaching does appear to have occured at a more rapid rate in the Weswood loam than in the Padina sand, and does not appear to have any correlation with the application rate. Thus, bacterial mutagens were detected in the leachate water from at least one lysimeter for each treatment where at least one pore volume of water was collected.

The weighted activity of the leachate water from the lysimeters defines the quantitative bacterial mutagenicity of the leachate water. The weighted activity is determined by multiplying the weight of solvent extractable organics in milligrams per liter (Table 6-6) by the specific activity in revertants per milligram (Table 6-7). This value provides an estimation of the quantitative mutagenicity of each leachate sample. The weighted activities observed in the leachate water from the sludge amended lysimeters ranged from below detection to 130 net revertants per liter in the 0.5 to 1.0 pore volume sample collected from the Padina sand which received two applications totalling 150 Mg/ha (Table 6-8). None of the 0 to 0.5 pore volume leachate samples induced a response that would be considered highly mutagenic. However, three of the 0.5 to 1.0 pore volume leachate samples induced a relatively high weighted activity. These include leachate samples from Replication 2 of the Padina sand treated with 150 Mg/ha, Replication 2 of the Padina sand treated with 50 Mg/ha and

Replication 2 of the Weswood loam treated with 150 Mg/ha. The response observed in these three samples ranged from 35 to 65 times the weighted activity of the composite leachate from the control lysimeters (Table 6-8).

TABLE 6.8. WEIGHTED ACTIVITY OF ACETONE EXTRACTABLE ORGANICS RECOVERED FROM LYSIMETER LEACHATE SAMPLES CONCENTRATED ON XAD COLUMNS

Lysimeter	Weighted Activity					
	0-0.5 PV		0.5-1.0 PV		1.0-1.5 PV	
	-S9	+S9	-S9	+S9	-S9	+S9
Background						
Padina	BD	0.2				
Weswood	2	2				
Rep. 1 (Padina	BD	17	94	BD	-	-
Rep. 2 150 Mg/ha)	55	26	132	43	-	-
Rep. 3	18	BD	14	64	-	-
Rep. 1 (Padina	-	-	-	-	-	-
Rep. 2 100 Mg/ha)	3	42	-	-	-	-
Rep. 3	BD	BD	-	-	-	-
Rep. 1 (Padina	70	BD	-	-	-	-
Rep. 2 50 Mg/ha)	73	48	218	96	-	-
Rep. 3	10	20	43	81	-	-
Rep. 1 (Weswood	89	114	190	159	-	-
Rep. 2 150 Mg/ha)	28	BD	244	80	352	149
Rep. 3	49	26	7	98	-	-
Rep. 1 (Weswood	247	354	203	179	-	-
Rep. 2 50 Mg/ha)	368	416	314	248	-	-
Rep. 3	300	200	169	80	315	163

Weighted activity in recertants per liter equals specific activity (revertants per mg) times residue weight (mg/L).
PV = Pore Volume; one half pore volume is equal to 47.5 liters.
Control values: DMSO (-S9) = 28; DMSO (+S9) = 36;
2-nitrofluorene (25µg/plate) = 1836; 2-aminoanthracene (+S9-10µg/plate) = 1397 total revertants. DB = Below detection.

These data indicate that municipal sludge amended soils may release mutagens from the zone of incorporation, and may pose a threat to groundwater if application rates and environmental conditions are not carefully monitored.

CONCLUSIONS

This portion of the research project was designed to monitor the environmental fate of sludge borne mutagens in a land application system. Following application to the soil, organic compounds in a municipal sludge may be degraded, volatilized into the atmosphere, leached into groundwater or runoff into surface water. Two major pathways for human exposure to mutagens in municipal sewage are plant uptake and leaching into groundwater. For a chemical or mixture of chemicals to be safely land applied, degradation should occur at a faster rate than mobilization.

The evaluation of the specific activity of the solvent extract of the sludge amended soils indicates that the sludges examined may contain a persistent mutagenic organic residue. The bacterial mutagenicity of the sludge amended soil was similar to the activity of the equivalent fraction of the sludge. That is, for the most part, direct acting mutagens were detected in the CH_2Cl_2 fraction, indirect acting mutagens were detected in the CH_3OH fraction, and both classes of mutagens were detected in the combined fraction. This mutagenic activity also appears to be persistent in the soil. Although some fluctuations were observed in the waste amended soil, the samples collected 175 days following waste application induced a response that was usually equal to or greater than the response observed in the sample collected immediately after waste application. Ultimately, at least one fraction extracted from each of the soil samples collected induced a positive response in the bacterial mutagenicity assay.

The specific activity of the soil core samples defines the qualitative bacterial mutagenicity of the solvent extract of the sludge amended soil. The specific activity of the sludge amended soil collected immediately after waste application ranged from 41 net revertants to 184 net revertants (Table 6-4). Following waste application, the specific activity in most cases was increased. The maximum specific activities for the three fractions extracted from the sludge amended soil were 297 net revertants for the CH_2Cl_2 fraction, 416 net revertants for a CH_3OH fraction, and 333 net revertants for a combined fraction extracted 154 days after waste application from the Weswood soil which received two sludge applications (Table 6-4). The majority of samples collected seven months following waste application induced a strong positive response in the bioassay. However, the specific activity of the combined fraction extracted from the Weswood soil which received 100 or 50 Mg/ha was only 49 and 59 net revertants, respectively (Table 6-4). For comparative purposes, the maximum specific activity of a wood preserving waste amended soil was 1561 net revertants (Donnelly _et_ _al_., 1987b). Thus, the half-life of the specific activity appears to be greater than at least 200 days.

The weighted activity of the sludge amended soil did, however, display a decrease with time, indicating that the quantity of mutagens in the sludge amended soil was reduced with time. The weighted activity of the combined fraction extracted from the sludge amended soil 207 days after application was 321 revertants per gram for the Padina soil, and 125 revertants per gram for the Weswood soil (Table 6-5). The maximum weighted activity of a wood preserving waste amended soil was 12028 revertants per gram (Donnelly _et al._, 1987b),while Brown _et al_. (1986) determined that the weighted activity of an unamended Weswood soil was 2 revertants per gram. For the Padina sand which received one sludge application at 50 Mg/ha the weighted activity of the fractions extracted from the sample collected 175 days following sludge application were approximately equal to the weighted activity of the equivalent fraction extracted from the unamended soil. Thus, at the lowest application rate to the Padina sand, the weighted activity of the soil returned to background levels approximately 175 days after sludge application, while in the soils which received 150 Mg/ha of sludge, the weighted activity of the combined fractions collected 207 days after application were approximately 87% and 25% the weighted activities of the fraction collected immediately following sludge application for the Padina and Weswood soil, respectively (Table 6-5).

The results from the analysis of the soil pore leachate samples can be used to define the potential for a release of water soluble organics from below the treatment zone (0 to 30 cm). A previous study by Brown _et al_. (1986) found that soil core samples were more appropriate for the detection of hydrophobic chemicals (compounds with an octanol:water (K_{ow}) coefficient >4), while soil pore samples were efficient for the detection of hydrophillic compounds (compounds with a K_{ow}<2). The specific activity of the leachate samples defines the qualitative bacterial mutagenicity of the samples tested. For example, a potent bacterial mutagen such as aflatoxin B_1 induced a response of 19,400 revertants per microgram, while banzo[a]pyrene induced a response of 137 revertants per microgram in strain TA98 (McCann _et_ _al_., 1975); for comparison, the maximum specific activity observed in a leachate sample was 131 net revertants per 10 micrograms (Table 6-7). The maximum specific activity of a runoff water from a Weswood soil amended with a wood preserving waste was 783 net revertants with metabolic activation 360 days after waste application (Davol _et_ _al_., 1988).

In the present study, appreciable quantities of mutagens were detected in several of the leachate samples. The specific activity of the leachate samples ranged from below detection to net revertants per 10 mg (Table 6-7). Nine of twenty-six leachate samples induced a doubling of revertants at the highest dose tested (Table 6-7). Two of the leachate samples collected from the lysimeters which received 150 Mg/ha of sludge induced a positive response. In addition, one of the samples collected from the Padina sand which received 50 Mg/ha induced a positive response. These data indicate that municipal sludge amended soils can release mutagens into the soil pore water.

The weighted activity of the leachate water from the lysimeters defines the quantitative bacterial mutagenicity of the leachate water. This value provides an estimation of the quantitative mutagenicity of each leachate sample. For comparative purposes, a runoff water sample from a Weswood soil amended with a wood preserving waste had a weighted activity of 241 net revertants per liter without activation, and 1997 net revertants with activation immediately following waste application (Brown and Donnelly, 1984). An earlier study by Grimm-Kibalo _et_ _al_.(1981) observed the weighted activity of drinking water to be 60 revertants per liter, while Van Kreijl *et al*. (1980) found the weighted activity of water from the Rhine River to be more than 300 revertants per liter. The weighted activities of the leachate samples collected from the sludge amended lysimeters ranged from below detection to 416 revertants per liter (Table 6-8). Twelve of twenty-six samples induced a weighted activity of greater than 100 revertants per gram, with four of those samples inducing a weighted activity of greater than 300 revertants per liter (Table 6-8). Thus, the weighted activity of the leachate samples was often more than twice that of drinking water, and occasionaly greater than the weighted activity of surface water contaminated with a wood preserving waste.

SECTION 7

PLANT UPTAKE OF MUTAGENS FROM SEWAGE SLUDGE AMENDED SOILS

Perhaps one of the least studied routes of exposure associated with land application of municipal sewage sludge is the translocation of organic chemicals from the soil to plants. Previous studies have determined that municipal sewage sludge contains numerous priority pollutants (Bridle and Weber et al., 1983; EPA, 1983) and that the majority of sludge samples tested contained detectable levels of organic mutagens (Hopke et al., 1982; Hopke et al., 1984; Tabor et al., 1984; Donnelly et al., 1987a; Johnston et al., 1983). While there is a large amount of data describing uptake and the translocation of inorganic compounds by plants (Smith et al.,1983), the translocation of organic chemicals into plants has been less well characterized.

A study by Fries et al. (1981) demonstrated that soybean plants were not contaminated with polychlorinated biphenyls (PCBs) through root uptake or translocation, but some foliar contamination from vapor sorption did occur. Iwata et al. (1974) examined the uptake of the PCB Aroclor 1254 from soil by carrots. They observed that small levels of PCB's were adsorbed by the carrot, with increasing uptake associated with decreasing chlorination. In addition, since 97% of the residue was found in the peel, they assumed that very little translocation occurred in the plant tissue (Iwata *et al*., 1974). Chou *et al*. (1978) studied the plant uptake of polybrominated biphenyls (PBBs) and observed no translocation of PBBs by corn and soybean seedlings. However, translocation of PBBs was demonstrated for root crops, with the extent of uptake related to the clay and organic matter content of the soils. All of the roots examined contained higher concentrations of PBB when grown in soil with a lower clay and organic matter content (Chou *et al*., 1982). Overcash (1986) studied the translocation of three chemical classes from soil and found that for the three classes, the bioaccumulation factor (concentration of chemical in the plant divided by the concentration of the chemical in the soil) was always less than 1.0, often less than 0.1, and the majority were less than 0.01. In this study plant uptake was governed largely by the loss of the chemical from the soil, and by the water solubility of the chemical (Overcash, 1986). They also noted that the addition of sewage sludge to the soil did not appear to alter significantly the uptake of organics from the soil (Overcash, 1986).

A study by Boyd *et al*. (1982) evaluated plant uptake of organics by cabbage, beets, green beans, and butternut squash grown on municipal sludge treated plots. The vegetables were fed as 25% of the diet of rats for 12 weeks. They observed no overt toxicological response in these rats. However, the results of mutagenesis testing with *S*. *typhimurium* indicated a positive dose response with the extracts of sludge-grown beans, and in the urine of the rats consuming the sludge-grown beans (Boyd *et al*., 1982). The toxicological effects of cabbage grown on municipal sewage sludge amended soils in guinea pigs was examined by Babish _et al_.(1979). They observed no signs of overt toxicity although elevated levels of polychlorinated biphenyls were detected in the leaves of the plants, and in the livers of the animals fed the plants grown on the sludge amended soil. A sample of anaerobically digested municipal sewage sludge was observed to induce point mutations in the germ cells of _Zea mays_ and cytogenetic damage in meiotic cells of _Tradescantia paludosa_ (Hopke _et al_., 1982). These studies indicate that mutagenic chemicals can be detected in plants grown on a sludge amended soil. In addition, the information in the literature suggests that plant uptake of organic mutagens from a sludge amended soil is directly related to a chemicals' water solubility, and inversly related to the rate of loss of the chemical from the soil, and the clay content of the soil.

The extraction and analysis of the plants grown on sludge amended soil will provide data to describe the potential for plant uptake of mutagens from soil. If the data indicate that there is a high potential for uptake, this would provide a principal pathway for transmisssion of mutagens through the food chain. Two studies were conducted to evaluate the potential for uptake of organic mutagens from sludge amended soil. One study evaluated the mutagenic potential of plants grown on sludge amended soil, while a second study was used to evaluate the interactions of known mutagens with the extract of plants grown on sludge amended and control soils.

MATERIALS AND METHODS

Chemicals

Chemicals employed in the plant uptake study were purchased from Sigma Chemical Co. (St. Louis, MO) and included benzo[a]pyrene (BAP), chlorophyllin, 3-methylchloranthene, 2-aminoanthracene, 2-nitrofluorene and B-glucosidase.

Sludge Samples

Two samples were collected for use in the plant uptake study. One sludge (SM 6) had been used in the earlier lysimeter study, and the second sludge sample (AD) was used only in this study. The treatment process employed for the first sludge has been described in Section 5 . The SM6 sludge sample was obtained from the C.E. Raymond cage mill flash dryer. The plant served a population of almost 300,000 and treats approximately 38 million gallons of wastewater each day, including approximately 20% from industries. The primary industries contributing to this plant are manufacturing.

Two sludge samples were collected, one each in September and October from a wastewater treatment plant in Illinois (AD1, AD2). Ellis *et al*. (1982) identified more than 300 manufacturing and industrial firms contributing to this system. The sludge samples were aerobically digested. The samples were collected from drying beds, and had a moisture content of 63%.

Soils

One soil was used to provide a coarse textured media capable of supporting vigorous plant growth. The A horizon of a Padina (formerly Lakeland) fine sand (Grossarenic Paleustalf) was collected from a pasture area adjacent to the Brazos River. The area from which this soil was collected had been used as a pasture for grazing cattle for many years prior to sample collection. Thus, the potential for chemical contamination is anticipated to be relatively low.

Greenhouse Boxes

Wooden boxes were built (0.45 m wide x 0.57 m long x 0.02 m high) and painted with an epoxy based heavy metal free paint (Shertar, Sherwin-Williams Co.). Drainage holes were placed in the bottom of each box to provide adequate aeration. The soil was air dried and sieved through a 4 mm mesh screen. Prior to the amendment of the sewage sludge, the soil-sludge mixture was packed in the boxes in 5 cm layers to a depth of 17 cm. Twenty-four boxes of each soil were prepared as described above. Sludge was applied to three replications of each application rate and the remaining three boxes served as controls. Sludge application rates included 22.4, 112, and 224 Mg/ha.

The amount of sludge applied to the soil in each box was based on the volume of soil (3.63×10^{-2} m^3) and the moisture content of the sludge. Thus, for the 22.4 Mg/ha application rate, the equivalent to 1.70 kg of sludge was applied to the soil in the boxes receiving that treatment; while, for the 112 and 224 Mg/ha application rates, 8.5 and 17 kg of sludge were applied, respectively.

A piece of sheet aluminum (450 cm x 20 cm) was placed in the center of each box to divide the box into two equal sections. Alfalfa (*Medicago* sativa) was seeded in one half of each box, while bermudagrass (Cynodon dactylon L.) was sprigged in the other half. Planting was done 1 or 2 days following sludge application.

Plant Uptake

The plant uptake study was divided into two experiments. One experiment was conducted to evaluate the mutagenic potential of the extract of plants grown on sludge amended and control soils, while a second experiment was conducted to attempt to investigate some of the interactions of known mutagens with various plant extracts.

In the first study, bermudagrass or alfalfa were grown on soil amended with either the AD2 sludge, or the SM6 sludge. Plant samples were collected 60, 122, and 152 days after sludge application. There were no plant samples collected from the two highest application rates for the SM6 sludge amended soil as the salinity and acidity of this sludge inhibited plant growth.

Plant Extraction

Plant samples were collected from each treatment on the sampling dates (60, 122, and 152 days after sludge application), and stored in a plastic bag in a freezer at 0°C. After the sample was removed from the plastic bag, it was cut into pieces approximately 0.5 cm long. The pieces were frozen in a mortar on a bed of dry ice, crushed to a powder using a mortar and pestle, and approximately 15 g were weighed into a precleaned cellulose thimble.

The thimbles containing the plant samples were extracted at 188°C using a Tecator extraction unit with 25 ml acetone. The samples were extracted by boiling for 5 min and rinsing for 50 min. The solvent was evaporated to a volume of less than 10 mL during the last 5 min of the cycle. The residue was transferred to a tarred test tube, and taken to dryness under a stream of nitrogen.

For the second sequential extraction, the heater unit was set at 200°C. methanol (25 mL) was added to the extraction cups, and the extraction repeated as described for the acetone extraction. The resulting residues were combined with the acetone extract and solvent exchanged into DMSO.

Bioassay Procedure

The *Salmonella*/microsome assay of Ames *et al*. (1975) was used to evaluate the mutagenicity of the solvent extractable organic compounds from the sludge, soil, or plant samples. The *Salmonella* strains were kindly supplied by Dr. B.N. Ames (University of California, Berkeley, CA). The procedural methods were the same as Ames *et al*. (1975) with the modifications suggested by Maron and Ames (1983). The Salmonella tester strain TA98 was utilized with and without metabolic activation. Only strain TA98 was used for the plant uptake study as the analysis of sewage sludge samples had indicated that this strain was the most sensitive to the types of mutagens anticipated. The S9 mixture (9,000 x G supernatent from homogenized liver) contained 0.3 mL rat-liver S9 and 0.7 mL cofactor supplement (11.4 mM $MgCl_2$, 47 mM KCl, 7.1 mM glucose-6-phosphate, 5.7 mM NADP and 140 mM potassium phosphate buffer at pH 7.4). Aroclor induced Sprague-Dawley rat liver was obtained from Organon Teknika (West Chester, PA). Each fraction obtained from the sludge, soil or plants was tested in the standard plate incorporation assay at a minimum of four dose levels 1.0, 2.5, 5.0, and 10.0 (mg/plate). The data were analyzed using the modified two-fold rule (Chu et al., 1981). Using this procedure, a response is considered positive if the average response for at least two consecutive dose levels was greater than twice the average response for the concurrent negative/solvent control (Chu et al., 1981). All strains were checked monthly for nutritional markers (histidine and biotin) and other genetic characteristics, i.e., sensitivity to crystal violet, ampicillin, and UV light. On each test date, all tester strains were calibrated with positive and negative controls. Positive controls included 25 µg/plate 2-nitrofluorene (Aldrich, Milwakee, WI). 2-Aminoanthracene (Sigma) at 5 µg/plate was used to verify the functioning of the metabolic activation system. All reagents and extracts were tested for sterility; dimethyl sulfoxide (DMSO, Grade I, Sigma) was used as a solvent control.

Pre-incubation procedure

Several of the plant extracts were also tested in a preincubation study. Matsushima et al. (1980) suggest that mutagens translocated into plants may exist in conjugated form such as glycosides. To detect bound residues, Matsushima et al. (1980) recommend preincubating plant extracts with B-glucosidase. This preincubation step should effectively cleave any bound residue and allow their detection in the microbial bioassay. To conduct the pre-incubation study, the bacteria and test chemical were added to a test tube along with a solution containing 50 units of B-glucosidase. The bacteria, test sample, and B-glucosidase were incubated for 30 minutes at 30°C with shaking as suggested by Matsushima et al. (1980). After the pre-incubation period, 2.5 mL of top agar was added to the tube, the contents were mixed on a vortex mixer, and plated on a minimal agar plate. The plates were incubated and scored as described for the standard plate incorporation assay.

RESULTS AND DISCUSSIONS

The data presented in Table 7-1 provides the dose-response and concurrent solvent control results for all of the plant extracts tested in the bioassay. These results indicate that none of the plant extracts tested induced a doubling of revertants at two or more consecutive dose levels. However, several of the plant extracts did induce an increased number of revertants when compared to the solvent control (Table 7-1). Thus, within the limits of this study, organic mutagens were not present at appreciable levels in the solvent extract of plants grown on municipal sludge amended soil.

Several of the plant extracts were also tested in a preincubation study to detect bound residues such as glycosides. The analysis of plant extracts from the present study using preincubation with B-glucosidase provided inconclusive information. None of the samples tested displayed a doubling of revertant colonies, and none showed even a dose related increase in the number of revertants (data not shown). Within the limitations of this study, there were no bound mutagenic residues present in the extracts of plants grown on sludge amended soil.

To evaluate the potential interactions of the naturally occurring organic compounds in the plant, which could mask the presence of mutagenic components of the sewage sludge, the extract of the AD2 sludge

TABLE 7-1. MUTAGENICITY, AS MEASURED WITH *S. TYPHIMURIUM* STRAIN TA98, OF SOLVENT EXTRACT OF PLANTS FROM CONTROL AND SLUDGE AMENDED SOILS

Treatment (Mg/ha)	Day of Harvest	Plant	Dose (mg/plate)	Total His^+ Revertants (Mean ± S.D.)† -S9	+S9
Control	60	Alfalfa	0	20 ± 4	36 ± 8
			1	19 ± 4	24 ± 1
			2.5	20 ± 4	30 ± 6
			5.0	42 ± 6	46 ± 14
		Bermuda	0	20 ± 4	36 ± 8
			1	20 ± 4	27 ± 0
			2.5	26 ± 0	36 ± 0
			5.0	26 ± 1	20 ± 6
			10.0	20 ± 8	NT
224	60	Alfalfa	0	20 ± 4	36 ± 8
AD1			1	19 ± 6	29 ± 6
			2.5	18 ± 5	24 ± 12
			5.0	24 ± 1	26 ± 0
			10.0	16 ± 1	30 ± 7
224	60	Alfalfa	0	20 ± 4	36 ± 8
AD1			1	22 ± 7	25 ± 1
			2.5	30 ± 5	28 ± 4
			5.0	38 ± 6	34 ± 2
			10.0	28 ± 6	31 ± 6
224	60	Bermuda	0	20 ± 4	36 ± 8
AD1			1	29 ± 8	26 ± 2
			2.5	24 ± 2	38 ± 5
			5.0	23 ± 0	38 ± 15
			10.0	20 ± 4	32 ± 8
22.4	122	Bermuda	0	21 ± 4	32 ± 8
AD1			1	27 ± 5	29 ± 6
			2.5	26 ± 1	36 ± 9
			5.0	24 ± 8	45 ± 18
			10.0	26 ± 14	54 ± 19

TABLE 7-1 CONTINUED

Treatment	Day of Harvest	Plant	Dose (mg/plate)	Total His$^+$ Revertants (Mean ± S.D.)† -S9	+S9
22.4	122	Bermuda	0	21 ± 4	32 ± 8
AD1			1	21 ± 5	34 ± 17
			2.5	21 ± 6	35 ± 12
			5.0	34 ± 14	43 ± 20
			10.0	29 ± 9	58 ± 12
112	122	Alfalfa	0	21 ± 4	32 ± 8
AD1			1	22 ± 4	32 ± 8
			2.5	22 ± 6	31 ± 6
			5.0	22 ± 5	34 ± 8
			10.0	33 ± 8	43 ± 10
112	122	Alfalfa	0	21 ± 4	32 ± 8
AD1			1	24 ± 6	31 ± 6
			2.5	30 ± 7	26 ± 5
			5.0	28 ± 9	25 ± 1
			10.0	30 ± 13	47 ± 14
112	122	Alfalfa	0	21 ± 4	32 ± 8
AD1			1	23 ± 4	34 ± 8
			2.5	24 ± 2	29 ± 4
			5.0	24 ± 5	35 ± 8
			10.0	29 ± 3	44 ± 4
112	122	Bermuda	0	21 ± 4	32 ± 8
AD1			1	21 ± 6	35 ± 1
			2.5	20 ± 6	28 ± 11
			5.0	30 ± 11	30 ± 6
			10.0	27 ± 5	43 ± 15
112	122	Bermuda	0	21 ± 4	32 ± 8
AD1			1	17 ± 3	34 ± 8
			2.5	23 ± 1	38 ± 5
			5.0	25 ± 8	33 ± 1
			10.0	24 ± 6	51 ± 13

TABLE 7-1 CONTINUED

Treatment (Mg/ha)	Day of Harvest	Plant	Dose (mg/plate	Total His$^+$ Revertants (Mean ± S.D.)† -S9	+S9
112 AD1	122	Bermuda	0	21 ± 4	32 ± 8
			1	23 ± 6	40 ± 12
			2.5	20 ± 9	40 ± 14
			5.0	24 ± 4	46 ± 11
			10.0	21 ± 4	48 ± 20
224 AD1	122	Alfalfa	0	21 ± 4	32 ± 8
			1	36 ± 7	27 ± 4
			2.5	38 ± 16	32 ± 6
			5.0	31 ± 13	30 ± 11
			10.0	31 ± 8	46 ± 8
224 AD1	122	Alfalfa	0	21 ± 4	32 ± 8
			1	30 ± 6	30 ± 6
			2.5	32 ± 10	32 ± 15
			5.0	35 ± 7	32 ± 4
			10.0	29 ± 7	44 ± 4
224 AD1	122	Alfalfa	0	21 ± 4	32 ± 8
			1	24 ± 9	29 ± 6
			2.5	25 ± 2	24 ± 3
			5.0	26 ± 8	30 ± 10
			10.0	29 ± 6	22 ± 4
224 AD1	122	Bermuda	0	21 ± 4	32 ± 8
			1	26 ± 9	26 ± 9
			2.5	24 ± 5	25 ± 9
			5.0	29 ± 5	33 ± 5
			10.0	26 ± 1	56 ± 9
224 AD1	122	Bermuda	0	21 ± 4	32 ± 8
			1	18 ± 3	32 ± 7
			2.5	22 ± 9	24 ± 3
			5.0	25 ± 8	30 ± 5
			10.0	24 ± 9	40 ± 6

TABLE 7-1 CONTINUED

				Total His$^+$ Revertants (Mean ± S.D.)†	
Treatment (Mg/ha)	Day of Harvest	Plant	Dose (mg/plate	-S9	+S9
224 AD1	122	Bermuda	0	21 ± 4	32 ± 8
			1	23 ± 4	31 ± 7
			2.5	22 ± 6	36 ± 1
			5.0	25 ± 9	50 ± 6
			10.0	38 ± 11	48 ± 5
Control	122	Bermuda	0	19 ± 4	32 ± 4
			1	30 ± 4	27 ± 0
			2.5	16 ± 1	31 ± 6
			5.0	34 ± 2	26 ± 0
			10.0	25 ± 7	25 ± 0
22.4 SM5	122	Alfalfa	0	19 ± 4	32 ± 4
			1	16 ± 1	39 ± 4
			2.5	24 ± 3	34 ± 1
			5.0	20 ± 6	25 ± 1
			10.0	24 ± 1	34 ± 11
22.4 SM5	122	Alfalfa	0	19 ± 4	32 ± 4
			1	16 ± 2	31 ± 5
			2.5	14 ± 0	31 ± 5
			5.0	20 ± 4	34 ± 2
			10.0	27 ± 0	33 ± 0
22.4 SM5	122	Alfalfa	0	19 ± 4	32 ± 4
			1	14 ± 0	26 ± 0
			2.5	20 ± 5	26 ± 0
			5.0	21 ± 5	34 ± 0
			10.0	22 ± 6	24 ± 0
22.4 SM5	122	Bermuda	0	19 ± 4	32 ± 4
			1	20 ± 0	14 ± 0
			2.5	19 ± 5	34 ± 0
			5.0	24 ± 5	20 ± 4
			10.0	24 ± 6	36 ± 2

TABLE 7-1 CONTINUED

Treatment (Mg/ha)	Day of Harvest	Plant	Dose (mg/plate	Total His$^+$ Revertants (Mean ± S.D.)† -S9	+S9
22.4 SM5	122	Bermuda	0	19 ± 4	32 ± 4
			1	20 ± 4	25 ± 1
			2.5	16 ± 4	30 ± 4
			5.0	21 ± 0	21 ± 4
			10.0	23 ± 9	30 ± 5
22.4 SM5	122	Bermuda	0	19 ± 4	32 ± 4
			1	20 ± 4	27 ± 4
			2.5	20 ± 4	26 ± 4
			5.0	24 ± 0	30 ± 0
			10.0	26 ± 9	28 ± 4
Control	152	Alfalfa	0	19 ± 4	32 ± 4
			1	21 ± 4	20 ± 6
			2.5	20 ± 5	16 ± 0
			5.0	18 ± 4	28 ± 5
			10.0	24 ± 1	30 ± 4
Control	152	Bermuda	0	19 ± 4	32 ± 4
			1	25 ± 10	32 ± 5
			2.5	19 ± 4	30 ± 6
			5.0	29 ± 3	25 ± 1
			10.0	20 ± 4	42 ± 0
Control	152	Alfalfa	0	19 ± 4	32 ± 4
			1	24 ± 2	25 ± 0
			2.5	20 ± 4	31 ± 6
			5.0	21 ± 4	24 ± 0
			10.0	16 ± 2	46 ± 0
Control	152	Bermuda	0	19 ± 4	32 ± 4
			1	20 ± 4	24 ± 1
			2.5	15 ± 1	25 ± 1
			5.0	18 ± 4	26 ± 0
			10.0	22 ± 0	27 ± 0

TABLE 7-1 CONTINUED

Treatment (Mg/ha)	Day of Harvest	Plant	Dose (mg/plate	Total His^+ Revertants (Mean ± S.D.)† S9	+S9
Control	152	Alfalfa	0	19 ± 4	32 ± 4
			1	20 ± 4	30 ± 4
			2.5	16 ± 2	24 ± 0
			5.0	22 ± 5	34 ± 11
			10.0	16 ± 1	40 ± 4
Control	152	Bermuda	0	19 ± 4	32 ± 4
			1	28 ± 6	30 ± 6
			2.5	29 ± 4	23 ± 0
			5.0	15 ± 0	29 ± 3
			10.0	24 ± 1	26 ± 8
Control	152	Alfalfa	0	20 ± 5	33 ± 5
			1	24 ± 2	27 ± 5
			2.5	20 ± 12	30 ± 5
			5.0	26 ± 1	28 ± 3
			10.0	19 ± 7	35 ± 7
Control	152	Bermuda	0	18 ± 4	31 ± 5
			1	21 ± 5	32 ± 5
			2.5	22 ± 4	34 ± 9
			5.0	29 ± 6	32 ± 4
			10.0	28 ± 6	38 ± 10
Control	152	Alfalfa	0	20 ± 5	33 ± 5
			1	21 ± 6	39 ± 7
			2.5	35 ± 1	26 ± 1
			5.0	20 ± 6	32 ± 1
			10.0	22 ± 1	40 ± 6
Control	152	Bermuda	0	18 ± 4	31 ± 5
			1	26 ± 10	35 ± 1
			2.5	18 ± 12	36 ± 1
			5.0	20 ± 10	36 ± 7
			10.0	19 ± 6	43 ± 5

TABLE 7-1 CONTINUED

Treatment (Mg/ha)	Day of Harvest	Plant	Dose (mg/plate	Total His$^+$ Revertants (Mean ± S.D.)† - S9	Total His$^+$ Revertants (Mean ± S.D.)† +S9
Control	152	Alfalfa	0	18 ± 4	31 ± 5
			1	16 ± 4	34 ± 1
			2.5	22 ± 5	38 ± 4
			5.0	22 ± 5	42 ± 10
			10.0	26 ± 4	45 ± 6
22.4 SM5	152	Alfalfa	0	20 ± 5	33 ± 5
			1	20 ± 6	41 ± 7
			2.5	18 ± 5	50 ± 6
			5.0	16 ± 0	43 ± 1
			10.0	24 ± 11	50 ± 5
22.4 SM5	152	Bermuda	0	22 ± 5	31 ± 5
			1	22 ± 5	32 ± 5
			2.5	20 ± 5	32 ± 8
			5.0	17 ± 3	40 ± 6
			10.0	24 ± 4	50 ± 15
22.4 SM5	152	Alfalfa	0	18 ± 4	31 ± 5
			1	22 ± 4	30 ± 6
			2.5	23 ± 5	39 ± 5
			5.0	17 ± 5	43 ± 10
			10.0	24 ± 2	41 ± 4
22.4 SM5	152	Bermuda	0	18 ± 4	31 ± 5
			1	33 ± 10	35 ± 1
			2.5	29 ± 12	36 ± 1
			5.0	28 ± 10	36 ± 7
			10.0	30 ± 6	43 ± 5
22.4 SM5	152	Alfalfa	0	18 ± 4	31 ± 5
			1	16 ± 4	34 ± 1
			2.5	22 ± 5	38 ± 4
			5.0	22 ± 5	42 ± 10
			10.0	26 ± 4	45 ± 6

TABLE 7-1 CONTINUED

Treatment (Mg/ha)	Day of Harvest	Plant	Dose (mg/plate	Total His^+ Revertants (Mean ± S.D.)† -S9	+S9
22.4 SM5	152	Bermuda	0	22 ± 5	31 ± 5
			1	19 ± 10	33 8
			2.5	18 ± 4	29 ± 12
			5.0	23 ± 10	32 ± 8
			10.0	21 ± 7	38 ± 10
224 AD1	152	Bermuda	0	19 ± 4	32 ± 4
			1	20 ± 4	24 ± 5
			2.5	21 ± 5	33 ± 4
			5.0	26 ± 4	26 ± 8
			10.0	27 ± 4	34 ± 1
224 AD1	152	Bermuda	0	19 ± 4	32 ± 4
			1	24 ± 5	26 ± 1
			2.5	18 ± 5	23 ± 5
			5.0	22 ± 4	30 ± 5
			10.0	24 ± 8	31 ± 3
224 AD1	152	Bermuda	0	19 ± 4	32 ± 4
			1	25 ± 8	30 ± 11
			2.5	19 ± 5	30 ± 6
			5.0	24 ± 12	23 ± 10
			10.0	22 ± 4	32 ± 9

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† Value represents mean ± standard deviation; obtained from duplicate plates in at least one independent experiment (N >2).

was mixed with three different concentrations of plant extract. The extracts were obtained from plants that were grown on sludge amended soil, or a control unamended soil. The results (Figure 7-1) show that the extract of plants grown on unamended and sludge amended soil may inhibit the mutagenic potential of the organic chemicals in sludge. The bacterial mutagenicity of the sludge organics was decreased or eliminated (Figure 7-1) when the organic extract of the sludge was mixed with the solvent extract of the plants grown on either unamended or sludge amended soil. At the highest dose of sludge extract tested (5 mg per plate), the addition of 1 mg of plant extract reduced the mutagenic response from 128 total revertants for the sludge, to 38 and 41 total revertants for the sludge with the extract from the sludge amended and control plant extracts, respectively (Table 7-2). The inhibition appears to have been slightly reduced when increasing amounts of plant extract were mixed with the sludge organics. In addition, there was no appreciable difference in the results obtained with the plant extract from the unamended or sludge amended soil. It is conceivable that this mechanism may be the cause of the absence of mutagenic activity observed in the extracts of the plants grown on the sludge amended soil.

In an attempt to evaluate the influence of chlorophyll on the mutagenicity of the control chemicals, tests were conducted using benzo[a]pyrene (BAP) and the extract of plants grown on sludge amended soil, as well as the extract of plants grown on sludge amended soil in the dark to reduce the quantity of chlorophyll in the plant. The results presented in Figure 7-2 and Table 7-2 indicate that the response of the

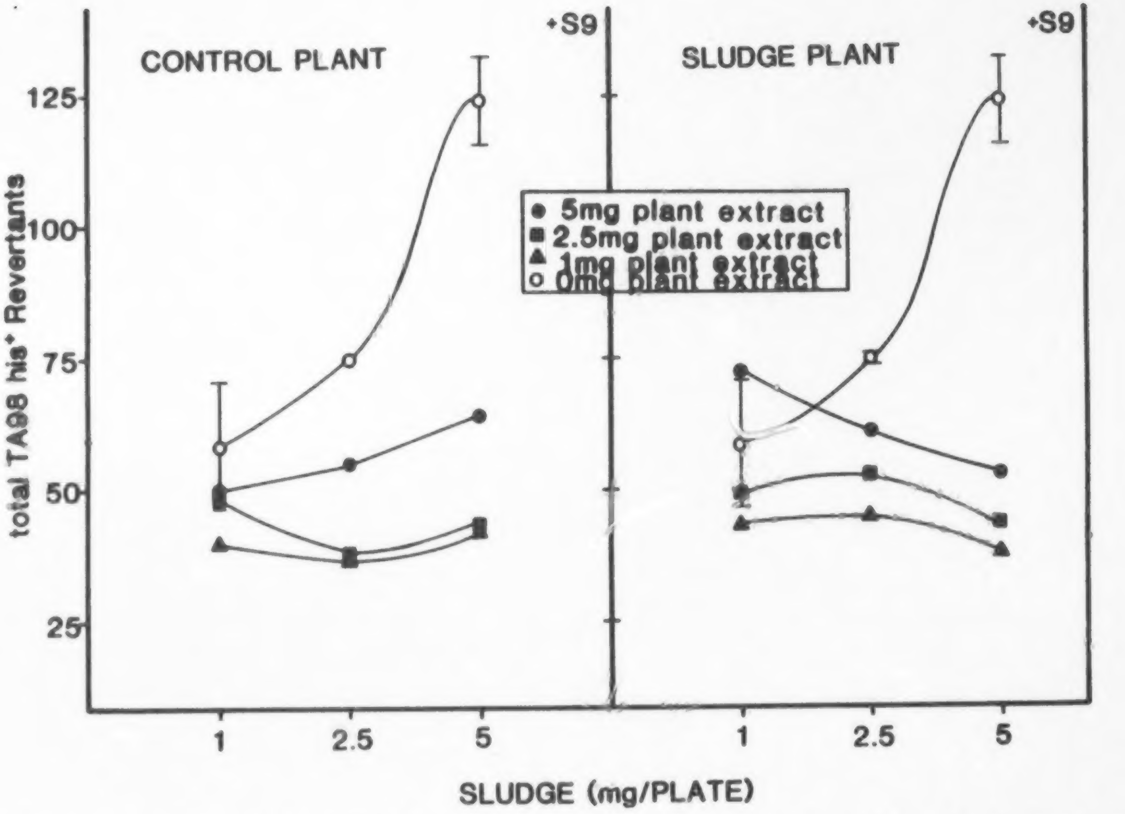

Figure 7-1. Mutagenic potential of municipal sludge extract when mixed with extract of plants grown on unamended or sludge amended soil. (Error bars represent one standard deviation).

TABLE 7-2. MUTAGENIC POTENTIAL OF MIXTURES OF POSITIVE CONTROLS WITH VARIOUS PLANT EXTRACTS

MIXTURES - BENZO[a]PYRENE AND PLANT EXTRACTS

LOW S9 (0.04 mg/mL)

BAP (mg/mL)	SLUDGE PLANT EXTRACT (mg/mL) 100	50	20	0
	-- Total TA98 his+ Revertants Per Plate --			
0.4	61 ± 6	41 ± 6	50 ± 20	118 ± 21
0.2	73 ± 15	70 ± 4	33 ± 1	134 ± 45
0.04	364 ± 28	238 ± 23	302 ± 55	44 ± 3
0.02	79 ± 24	137 ± 24	178 ± 15	44 ± 3
0.0	31 ± 5	23 ± 5	29 ± 5	25 ± 1

MEDIUM S9 (0.1 mg/mL)

BAP (mg/mL)	SLUDGE PLANT EXTRACT (mg/mL) 100	50	20	0
	-- Total TA98 his+ Revertants Per Plate --			
0.4	65 ± 17	35 ± 8	37 ± 8	113 ± 39
0.2	62 ± 29	39 ± 5	36 ± 1	60 ± 23
0.04	75 ± 1	40 ± 7	37 ± 6	34 ± 2
0.02	114 ± 11	72 ± 6	56 ± 6	30 ± 13
0.0	35 ± 1	35 ± 2	33 ± 6	19 ± 4

HIGH S9 (0.3 mg/mL)

BAP (mg/mL)	SLUDGE PLANT EXTRACT (mg/mL) 100	50	20	0
	-- Total TA98 his+ Revertants Per Plate --			
0.4	238 ± 22	143 ± 24	45 ± 19	167 ± 17
0.2	336 ± 26	271 ± 15	167 ± 5	74 ± 26
0.04	265 ± 28	126 ± 43	84 ± 42	42 ± 6
0.02	273 ± 35	167 ± 40	65 ± 8	52 ± 6
0.0	27 ± 8	27 ± 0	25 ± 10	34 ± 9

TABLE 7-2 CONTINUED.

MIXTURES - BENZO[a]PYRENE AND CHLOROPHYLLIN

LOW S9 (0.04 mg/mL)

BAP (mg/mL)	CHLOROPHYLLIN (mg/mL)				
	5	2	1	0.1	0
	Total TA98 his+ Revertants Per Plate				
0.4	45 ± 0	39 ± 6	31 ± 6	39 ± 13	118 ± 21
0.2	93 ± 9	69 ± 16	162 ± 30	46 ± 1	134 ± 45
0.04	94 ± 2	87 ± 12	149 ± 40	45 ± 11	44 ± 3
0.02	66 ± 19	95 ± 27	183 ± 12	56 ± 11	44 ± 3
0.0	30 ± 4	29 ± 7	22 ± 6	22 ± 5	25 ± 1

MEDIUM S9 (0.1 mg/mL)

BAP (mg/mL)	CHLOROPHYLLIN (mg/mL)				
	5	2	1	0.1	0
	Total TA98 his+ Revertants Per Plate				
0.4	286 ± 49	260 ± 36	195 ± 51	58 ± 6	113 ± 39
0.2	160 ± 32	237 ± 39	211 ± 64	64 ± 21	60 ± 23
0.04	167 ± 16	321 ± 14	168 ± 7	47 ± 8	34 ± 2
0.02	169 ± 26	301 ± 7	137 ± 24	36 ± 5	30 ± 13
0.0	29 ± 7	26 ± 11	29 ± 14	27 ± 6	19 ± 4

HIGH S9 (0.3 mg/mL)

BAP (mg/mL)	CHLOROPHYLLIN (mg/mL)				
	5	2	1	0.1	0
	Total TA98 his+ Revertants Per Plate				
0.4	269 ± 24	102 ± 11	51 ± 4	42 ± 4	167 ± 17
0.2	375 37	259 ± 25	159 ± 21	50 ± 10	74 ± 26
0.04	303 ± 30	219 ± 81	138 ± 21	59 ± 7	42 ± 6
0.02	308 ± 30	188 ± 100	82 ± 23	42 ± 5	52 ± 6
0.0	33 ± 5	21 ± 5	30 ± 5	36 ± 5	34 ± 9

TABLE 7-2 CONTINUED

MIXTURES - 2-NITROFLUORENE AND PLANT EXTRACTS

-S9

2NF	PLANT EXTRACTS (mg/mL) 100	50	20	0
(mg/mL)	Total TA98 his+ Revertants Per Plate			
2.0	2864 ± 127	2875 ± 137	2868 ± 215	3077 ± 223
1.0	2598 ± 126	2725 ± 107	2850 ± 144	3041 ± 65
0.5	2378 ± 108	2438 ± 58	2604 ± 171	2656 ± 250
0.02	188 ± 33	265 ± 37	288 ± 38	262 ± 54
0.0	19 ± 5	29 ± 7	27 ± 11	21 ± 5

+S9 (0.3 mg/mL)

2NF	PLANT EXTRACTS (mg/mL) 100	50	20	0
(mg/mL)	Total TA98 his+ Revertants Per Plate			
2.0	1786 ± 71	1892 ± 224	2044 ± 134	2086 ± 226
1.0	1248 ± 185	1195 ± 52	1167 ± 63	1375 ± 95
0.5	763 ± 1	639 ± 129	551 ± 30	622 ± 94
0.02	58 ± 11	47 ± 7	45 ± 10	48 ± 7
0.0	32 ± 5	24 ± 1	24 ± 10	24 ± 2

TABLE 7-2 CONTINUED

MIXTURES - BENZO[a]PYRENE AND PLANT EXTRACTS

------------------------ +S9 (0.3 mg/mL) --------------------

SLUDGE PLANT EXTRACTS (mg/mL)

----- Total TA98 his+ Revertants Per Plate -----

BAP (mg/mL)	100	50	20	0
0.4	101 ± 10	98 ± 15	101 ± 37	135 ± 34
0.2	116 ± 19	281 ± 5	334 ± 103	225 ± 102
0.04	236 ± 16	259 ± 15	295 ± 88	62 ± 15
0.02	66 ± 1	53 ± 24	40 ± 5	64 ± 18
0.0	32 ± 5	24 ± 1	24 ± 10	24 ± 2

--------------------- +S9 (0.3 mg/mL) ------------------------

ALBINO PLANT EXTRACTS (mg/mL)

---- Total TA98 his+ Revertants Per Plate ----

BAP (mg/mL)	100	50	20	0
0.4	134 ± 20	113 ± 16	119 ± 36	135 ± 34
0.2	298 ± 92	138 ± 26	170 ± 70	225 ± 102
0.04	201 ± 20	268 ± 30	208 ± 73	62 ± 19
0.02	59 ± 11	41 ± 6	41 ± 4	64 ± 18
0.0	32 ± 10	32 ± 6	35 ± 1	29 ± 5

--------------------- +S9 (0.3 mg/mL) ------------------------

CONTROL PLANT EXTRACTS (mg/mL)

---- Total TA98 his+ Revertants Per Plate ----

BAP (mg/mL)	100	50	20	0
0.4	202 ± 5	155 ± 35	97 ± 25	167 ± 17
0.2	183 ± 35	167 ± 41	110 ± 30	74 ± 26
0.04	212 ± 12	114 ± 46	139 ± 20	42 ± 6
0.02	251 ± 21	160 ± 35	89 ± 49	52 ± 6
0.0	29 ± 9	24 ± 8	25 ± 7	34 ± 9

TABLE 7-2 CONTINUED

MIXTURES - 2-AMINOANTHRACENE AND PLANT EXTRACTS

--------------------- +S9 (0.3 mg/mL) ---------------------

SLUDGE PLANT EXTRACTS (mg/mL)

2AA (mg/mL)	100	50	20	0
	-- Total TA98 his+ Revertants Per Plate --			
0.4	1961 ± 55	1939 ± 122	1652 ± 161	587 ± 123
0.2	324 ± 77	341 ± 130	328 ± 16	351 ± 67
0.04	239 ± 15	137 ± 15	148 ± 11	63 ± 11
0.02	66 ± 10	41 ± 11	35 ± 1	43 ± 15
0.0	32 ± 5	24 ± 1	24 ± 10	24 ± 2

---------------------- +S9 (0.3 mg/mL) --------------

ALBINO PLANT EXTRACTS (mg/mL)

2AA (mg/mL)	100	50	20	0
	-- Total TA98 his+ Revertants Per Plate --			
0.4	447 ± 49	656 ± 78	831 ± 57	587 ± 123
0.2	246 ± 35	254 ± 86	219 ± 20	351 ± 67
0.04	92 ± 26	155 ± 28	123 ± 41	63 ± 11
0.02	158 ± 50	35 ± 3	-	43 ± 15
0.0	32 ± 10	32 ± 6	35 ± 1	30 ± 8

TABLE 7-2 CONTINUED

MIXTURES - SLUDGE AND PLANT EXTRACTS

---------------------- +S9 (0.3 mg/mL) ----------------------

Sludge (mg/mL)	CONTROL PLANT EXTRACTS (mg/mL) 100	50	20	0
	-- Total TA98 his+ Revertants Per Plate --			
100	64 ± 10	43 ± 1	41 ± 6	124 ± 8
50	55 ± 19	38 ± 6	36 ± 10	75 ± 1
20	50 ± 5	48 ± 13	40 ± 6	58 ± 12
0	29 ± 9	24 ± 8	25 ± 7	34 ± 9

------------------ +S9 (0.3 mg/mL) ------------------

Sludge (mg/mL)	SLUDGE PLANT EXTRACTS (mg/mL) 100	50	20	0
	--- Total TA98 his+ Revertants Per Plate --			
100	54 ± 9	43 ± 6	38 ± 6	124 ± 8
50	61 ± 5	53 ± 10	45 ± 3	75 ± 1
20	72 ± 14	49 ± 4	43 ± 1	58 ± 12
0	27 ± 8	27 ± 0	25 ± 10	34 ± 9

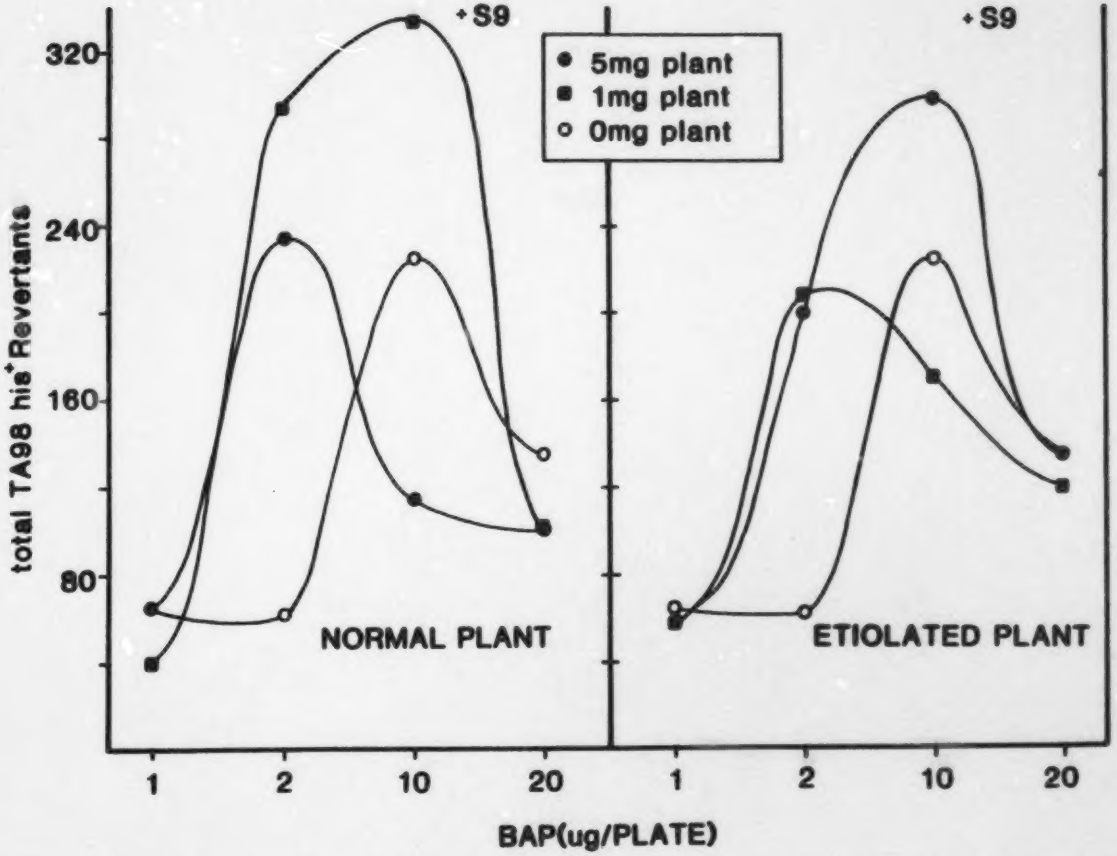

Figure 7-2. Mutagenic potential of benzo(z)pyrene when mixed with the extract of plants grown on sludge amended soil. (Error bars represent one standard deviation).

etiolated plant and the plant grown in sunlight were not appreciably different. Although the response induced by BAP and the BAP plant extract mixtures were very similar at the lower dose levels and at the highest dose, there was increased enhancement produced by the extract of the normal plant when compared to the response induced by the etiolated plant at a dose of 10 μg BAP per plate (Figure 7-2). Benzo(a)pyrene alone induced 225 total revertants at a dose of 10 μg per plate, while BAP plus 1 mg of plant extract per plate induced 334 total revertants (Table 7-2). The maximum level of enhancement for both the normal and etiolated plant extracts occurred at a BAP concentration of 2 μg per plate where the mutagenic potential of the mixture was three to four times greater than that of the BAP alone (Table 7-2). Thus, the extract of plants grown on sludge amended soil enhanced the mutagenic potential of BAP.

When a similar test was conducted with 2-aminoanthracene and the extracts of sludge plants and sludge plants grown in the absence of sunlight (etiolated plants), the enhancement effect was appreciably reduced in the presence of the extract of the albino plant. At the three lowest dose levels, the mutagenic potential of 2-aminoanthracene was not appreciably affected with either the etiolated plant or the sludge plant (Figure 7-3). However, at a dose of 20 μg per plate of 2-aminoanthracene, the mutagenic potential of the pure compound was 587 total revertants, while the mutagenic potential in the presence of the extract of the sludge plant grown in sunlight was increased approximately three-fold to 1961 total revertants (Table 7-2). The data obtained from testing the indirect acting mutagens benzo(a)pyrene and 2-aminoanthracene indicate that highly

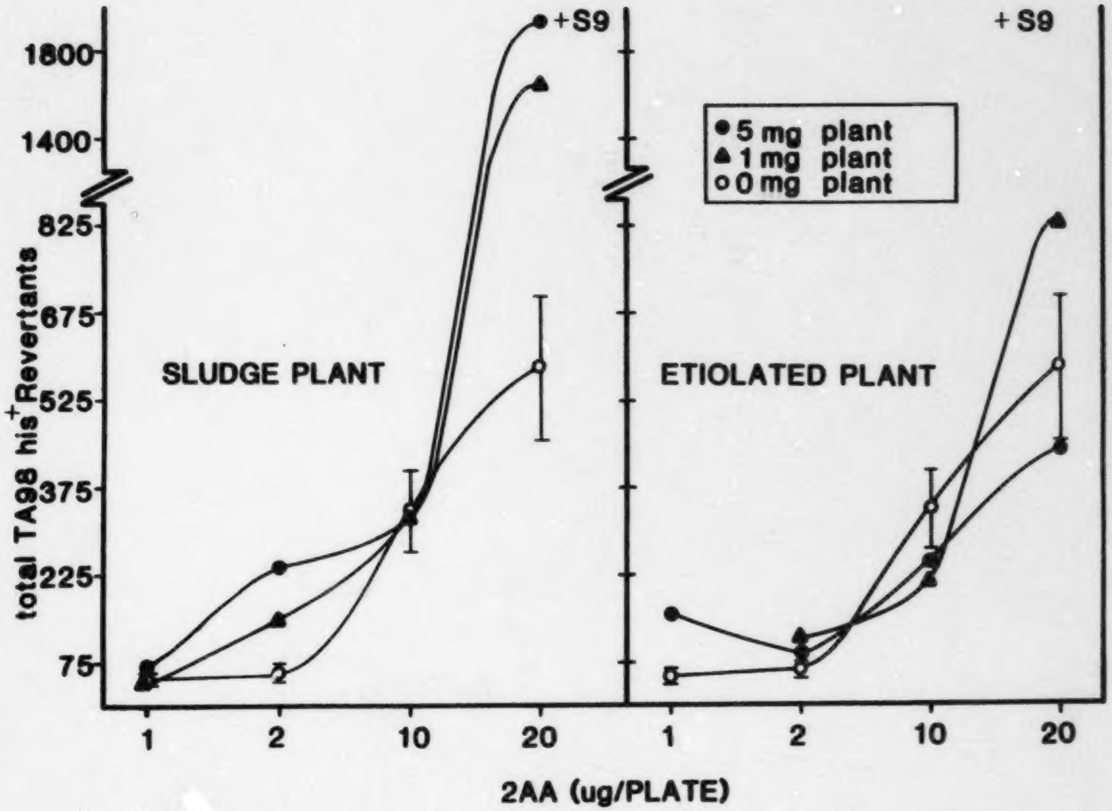

Figure 7-3. Mutagenic potential of 2-aminoanthracene when mixed with the extract of plants grown on sludge amended soil. (Error bars represent one standard deviation).

variable interactions may occur with mixtures of mutagens and the extract of plants. The predominant effect of the plant extract was to enhance the mutagenic potential of the organic mutagen, although additive and inhibitive responses were also observed.

The direct acting mutagen 2-nitrofluorene was also tested in binary mixtures with the plant extracts. The response obtained from the 2-nitrofluorene-plant mixtures was not appreciably different from the response obtained for 2-nitrofluorene alone (Table 7-2). The results suggest that since the indirect acting mutagens displayed strong interactions and the direct acting mutagens did not, the plant extract may influences the metabolic activation of the indirect acting mutagens only.

When the influence of different levels of metabolic activation were studied with the plant extract-BAP mixtures, the primary interaction observed was enhancement. With the low level of metabolic activation, enhancement was observed at the two lowest doses of BAP, while there was no appreciable difference in the response observed for the pure compound and the mixtures at the two highest dose levels of BAP. With the high level of metabolic activation, inhibition was observed for the highest dose of BAP with 1,000 µg per plate of plant extract, while the lower doses were not appreciably different from the pure compound. At a dose of 5,000 µg per plate of the plant extract with the high level of metabolic activation, the response observed was almost identical to that observed for BAP with 250 µg per plate chlorophyllin (Figures 7-2 and 7-4, respectively). At the lowest dose of BAP, the addition of 5,000 µg of plant extract increased the observed mutagenic response from 18 net revertants for the pure compound to

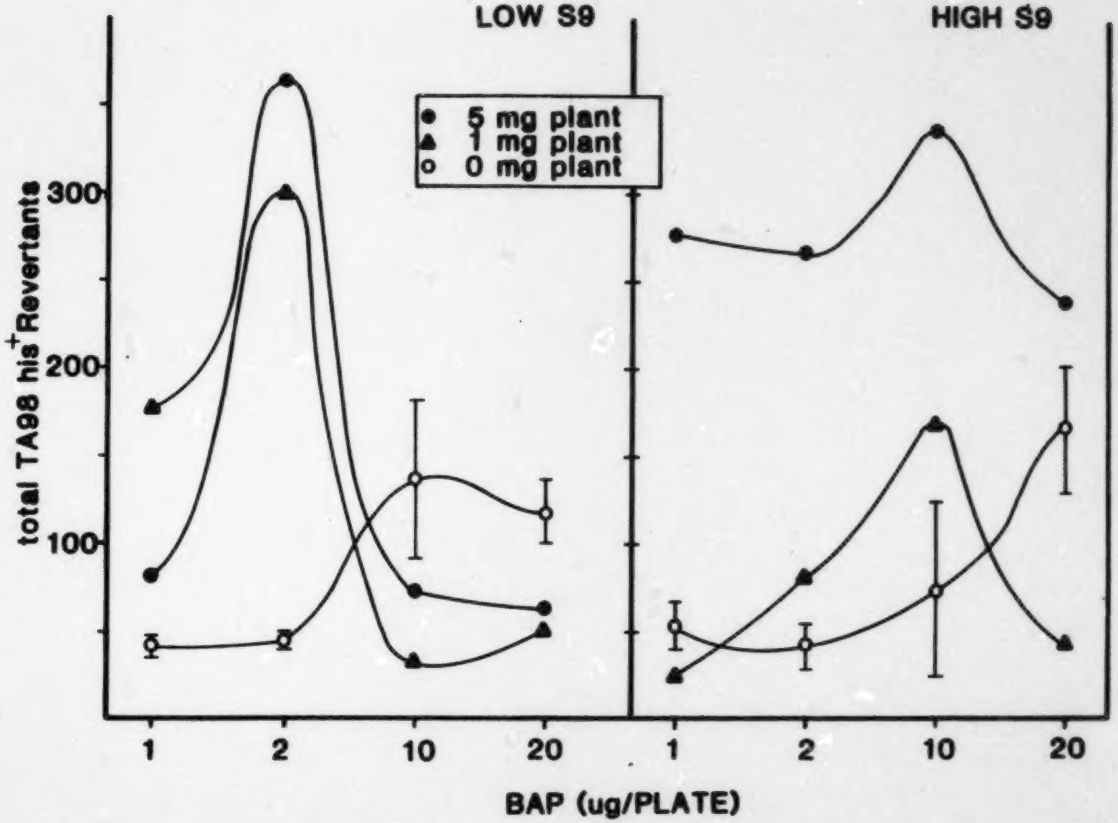

Figure 7-4. Mutagenic potential of benzo(a)pyrene, as measured with low (.04 mL S9/mL S9 mix) and high (0.30 mL S9/mL S9 mix) levels of metabolic activation, when mixed with the extract of plants grown on sludge amended soil. (Error bars represent one standard deviation.

246 net revertants for the compound plus the plant extract (Figure 7-4).

A separate study was conducted to evaluate the influence of chlorophyllin on the mutagenic potential of several known mutagens. This study was undertaken to provide information which could be used to determine if the absence of activity in the plant extracts was due to the absence of mutagens in the plant, or a result of the inhibition of mutagens by naturally occuring plant substances. Lai et al. (1980) observed that chlorophyllin or the extract of chlorophyll containing plants could inhibit the bacterial mutagenicity of benzo(a)pyrene and 3-methylchloranthene. The results presented in Figure 7-5 indicate that chlorophyllin served to enhance the mutagenic potential of the low dose levels of benzo[a]pyrene (BAP). The enhancement effect was greatest at the high level of metabolic activation, and was also observed to increase with increasing dose of chlorophyllin (Figure 7-5). At both the high and low levels of S9, the enhancement was eliminated when chlorophylin was tested with 20 µg per plate BAP (Figure 7-5). At the medium level of S9, enhancement was also observed, with the level of enhancement increasing at increasing dose levels of chlorophyllin (Table 7-5). The data also indicate that the enhancement effect observed with chlorophyllin was greatly reduced when compared to benzo[a]pyrene tested in mixture with either of the plant extracts. Whereas the bacterial mutagenicity with chlorophyllin was increased approximately three-fold, the bacterial mutagenicity of BAP with the extracts of the albino or sludge grown plant was only increased by approximately 30% (Figures 7-2 and 7-5). In addition, there does not appear to be a dose-related increase in the enhancement for the plant

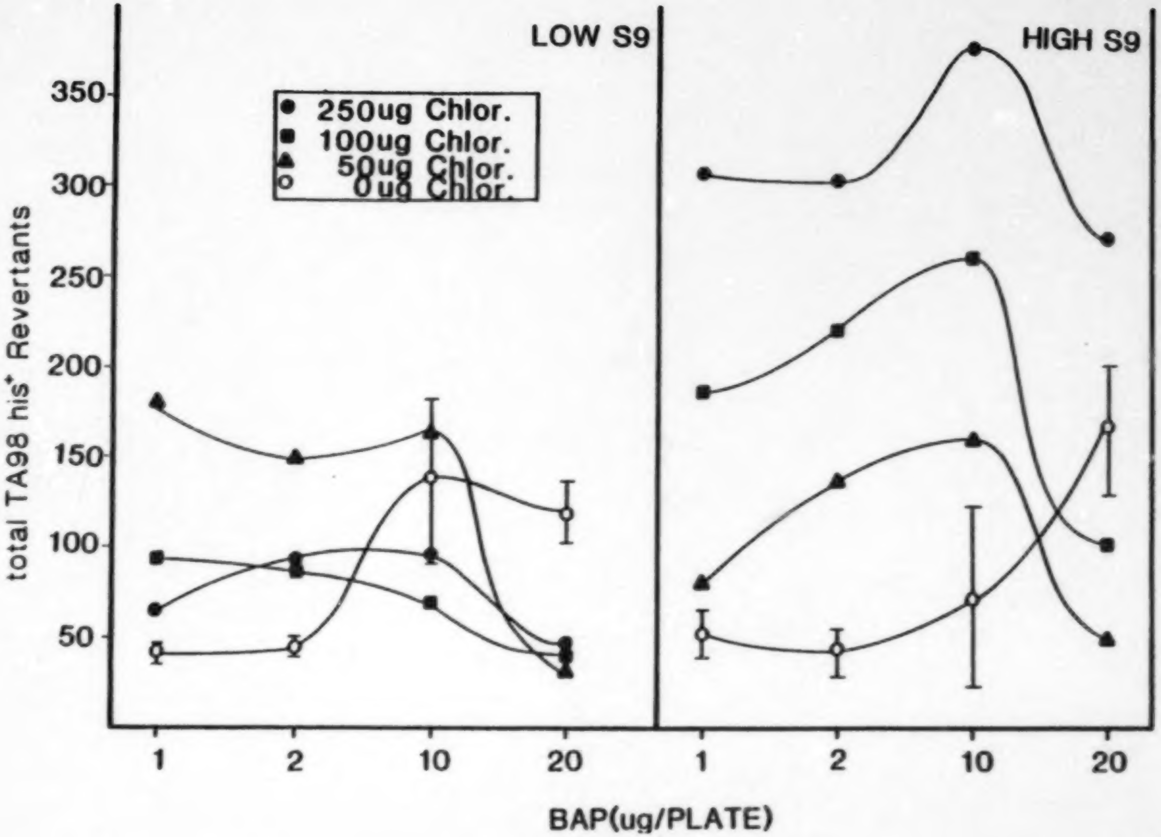

Figure 7-5. Mutagenic potential of benzo(a)pyrene, as measured with low (.04 mL S9/mL S9 mix) and high (0.30 mL S9/mL S9 mix) levels of metabolic activation, when mixed with chlorophyllin. (Error bars represent one standard deviation).

extracts, although a clear dose-related increase was observed for the mixture of BAP and chlorophyllin (Figure 7-5). The data obtained from testing a binary mixture of benzo(a)pyrene and chlorophyllin indicate that chlorophyllin enhanced the mutagenic potential of BAP at the majority of the dose levels tested.

CONCLUSIONS

For the sludges, soils, and plants employed for this study, there does not appear to be a significant potential for transmission of mutagens to the food chain through plants. The results indicate that at all dose levels tested, none of the extracts of plants grown on sludge amended soil induced a positive response in the *Salmonella* mutagenicity assay. Although the results indicate that mutagens were not detected in the plant extracts, this certainly does not indicate that plants will not absorb mutagens from sewage sludge amended soils.

These results suggest that there were no water soluble mutagens in the soil or that mutagens were not expressed; however, the data from the analysis of leachate samples indicate that mutagens were detected in the leachate water from the sludge amended lysimeters (Section 6). In addition, the analysis of the sludge amended soil in which the plants were grown indicates that mutagens were present in the majority of the soil samples tested (data not shown). Thus, although the results indicate that organic mutagens were not detected in the plant extract, it is possible that uptake would have been observed had we used different sludge samples, soils, or different plants.

The lack of bacterial mutagenicity in the plant extracts may have resulted from several very different situations. First, it is possible that mutagens were not taken up by plants at levels which could be detected by the bioassays. This may have been due to the types of sludges employed, the plants studied, or the extraction procedures used. The extent of translocation may have been limited until sufficient biodegradation had occurred to render the organic mutagens water soluble. The results of the lysimeter study indicate that this should require from eight to twenty weeks. Thus, it may be that sufficient time was not allowed to detect plant uptake. In addition, the presence of drainage holes in the greenhouse boxes may have permitted sufficient leaching of water soluble mutagens to limit the extent of translocation.

Second, it is possible that mutagens were incorporated into the plants, but plant constituents effectively inhibited the activity of the translocated mutagens. This is a distinct possibility, as the solvent extract of the plants served to inhibit the activity of the mutagens extracted from the sludge. Studies conducted using known mutagens and the extracts of plants grown on sludge amended soil further indicate that the results may have been complicated by synergism or antagonism of mutagens by the naturally occuring components of plants. Finally, the specific compounds translocated by the plants may have been genotoxic, but not mutagenic in the bacterial assay. This is not very likely as the extract of the sludge contained appreciable quantities of bacterial mutagens as did the sludge amended soil. However, conflicting results were obtained when the sludge extracts were tested with the plant extracts.

The data obtained in this study appear to conflict with that in the literature regarding the mutagenic potential of plants grown on sludge amended soil. Both Hopke et al., (1984) and Boyd et al. (1982) detected mutagens in the plants grown on sludge amended soil. The study conducted by Boyd et al. (1982) describes a mutagenic response in plants grown on sludge amended soil and in the urine from rats fed sludge-grown plants. However, the results presented in their manuscript do not include solvent control values, and the data that is provided indicate that using the modified two-fold rule (Chu et al., 1981) there is no doubling of revertant colonies at two consecutive dose levels. Thus, using the criteria used to evaluate the data from the present study, there were no positive responses induced by the plant extracts in the study of Boyd et al. (1982). In a separate study by Hopke et al. (1984), substantial uptake of mutagens was observed from a Chicago sludge. In addition, the whole or diluted sludges from Chicago or Sauget induced chromosome aberrations in meiotic cells of *Tradescantia* (Hopke et al., 1984). While these studies indicate that mutagens may be translocated by plants, the application rate employed in the study by Hopke et al. (1984) was substantially higher than those employed in the present study. In addition, the genotoxic endpoint detected in the *Tradescantia* assay is different from that detected in the *Salmonella* assay.

The data from the literature and from the present study indicate that the potential for plant uptake of mutagens is limited. Data from the present study and that of Boyd et al. (1982) indicate that the mutagenic potential of plants grown on sludge amended soil is likely to be relatively low. In addition, the results of Hopke et al. (1984) indicate that substantial uptake of mutagens can occur when excessive sludge application rates are utilized. Information in the literature indicate that plant uptake will largely be governed by a chemical's water solubility, rate of biodegradation, and the clay content of the soil. Thus, the major health threat of plant uptake of mutagens is likely to be associated with the application of an unusually toxic sludge, unusually high concentrations of sludge, or a sludge which had been weathered and contained significant quantities of water soluble mutagens.

SECTION 8

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